Testing technosols over an ultramafic gradient for rehabilitation of diamond mine wastes in a subarctic region

by

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Abstract

Subarctic regions are recognized as one of the largest remaining pristine landscapes on earth, but are currently experiencing progressive degradation due to human activity. Mining and site rehabilitation in northern regions can be challenging largely due to the remote location of these developments. Rehabilitation is often restricted to using local mine waste materials to construct technosols. This study focused on how mines in northern regions, specifically the isolated De Beers Victor Diamond Mine, can use local mineral mine waste material to manufacture successful soil covers. Specifically, our research focused on examining the performance and success of technosols across a gradient increasing in serpentine characteristics. The objectives of the study were to, (1) determine how technosols increasing in serpentine characteristics influence vegetation establishment and microbial activity, (2) determine how the quantity of peat influences vegetation establishment and microbial activity, and (3) determine how early physical and chemical characteristics, and early plant colonization on our technosols compare to a local natural environment undergoing primary succession. We constructed a factorial experiment on two areas of mine waste at the Victor Mine, the waste rock dumpsite, and a processed kimberlite storage facility. Three test blocks were constructed on each site comprising a total of 48 5 m x 5 m experimental plots with four mineral substrate mixtures, and two levels of peat application. Mixtures consisted of various combinations of coarse and fine processed kimberlite, a silty loam marine overburden, and 20% or 40% peat, creating a gradient with ultramafic, serpentine characteristics. Each plot was fertilized at a rate of 12.5 g m^{-2} (NPK 8-32-16), inoculated with a microbial inoculation 'tea' mixture, and seeded with a variety of native vegetation. Various early physical, chemical and biological characteristics were examined, including early plant colonization and microbial activity. To measure microbial activity, a 7-day aerobic soil incubation experiment was preformed in growth chambers, with and without the addition of glucose, where CO_2 respiration rate was the only parameter examined. The results indicated that mixtures increasing in serpentine characteristics resulted in decreased vegetation establishment and microbial activity in the short-term, while the mixtures that were non-serpentine (100% overburden-peat) were the most successful for vegetation establishment and potentially short-term microbial activity. However, most differences between our mixtures were minor. Differences in quantity of peat were minor between our experimental mixtures, causing them to have no influence on the vegetation establishment between our mixtures, and only minor differences in microbial activity between our mixtures. Our technosols shared similar physical and chemical aspects with the early successional Attawapiskat River floodplain environment, and also shared similarities with a peat-overburden mixture currently being used to rehabilitate a stockpile at the Victor Mine. These early similarities could show their potential for success during mine rehabilitation. Our research provides insight into challenges associated with rehabilitation of subarctic environments and, in general, mine waste substrates. This research will provide the De Beers Victor Mine with suggestions for rehabilitation upon closure, and will provide them with information on challenges they may face if incorporating processed kimberlite into technosols.

Key words: serpentine, processed kimberlite, primary succession, mine reclamation, microorganisms

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CHAPTER 1: General Introduction

With increasing global demand for resources, potential for mining in northern regions has increased (Bradshaw & Chadwick, 1980; Elberling *et al.*, 2007). Subarctic regions are recognized as one of the largest remaining pristine landscapes on earth (Walker, 1996), and are currently experiencing progressive degradation due to human activity (Deshaies *et al.*, 2009), leading to more demand for site rehabilitation. Both site rehabilitation and mining in northern regions can be challenging due to natural aspects of the environmental such as short growing seasons, nutrient deficient soils, and challenging microclimates (Johnson & Van Cleve, 1976; Riley, 2003; Abraham & Keddy, 2005). The remote location of these new mining developments also pose a challenge for both development and rehabilitation efforts. Often, there are limited materials available to create new soils, termed technosols, post-closure, and it is not ideal to transport external materials to these northern mining developments due to cost. Therefore, it is important to explore how mining developments located in northern regions can efficiently use material readily available to them, including their mine waste substrates, to manufacture successful technosols for the mine rehabilitation process.

Research has been conducted on northern Alberta's Athabasca Oil Sands mining developments that focus on using local peat-mineral mixtures for rehabilitation (Danielson *et al.*, 1983; Moskal *et al.*, 2001; McMillan *et al.*, 2007; Rowland *et al.*, 2009; Turcotte *et al.*, 2009). Mineral mine waste substrates incorporated into technosols are capable of influencing their physical, chemical, and biological properties (Danielson *et al.*, 1983; Reid & Naeth, 2005a, 2005b; Drozdowski *et al.*, 2012; Naeth & Wilkinson, 2014). The success of establishing native plant and microbial communities can depend largely on chemical aspects of mine waste parent materials. For example, diamond mining produces waste deposits that are ultramafic due to the kimberlite parent material. Ultramafic refers to igneous or metamorphic parent material that contains an abundance of Mg and low amounts of silica (Kruckeberg, 2002). In the natural environment soils formed from ultramafic material are referred to as serpentine soils, and can be a challenging environment for colonization (Brooks, 1987 as cited by Brady *et al.*, 2005). Therefore, technosols incorporating ultramafic parent material can possess similar qualities (Reid & Naeth, 2005a, 2005b; Hobbs *et al.*, 2007). Serpentine soils can be used as a comparison for the potential performance and success of ultramafic soil covers. This topic will be discussed with more detail in Chapters 2 and 3.

Our research focuses on how diamond mines in northern regions can effectively use available mineral mine waste and organic substrates to manufacture technosols for rehabilitation. We constructed technosols using various materials at the De Beers Victor Diamond Mine, in Ontario's Far North. The objectives of our study were to (1) determine which mixtures of mineral mine waste substrates promote better conditions for plant and microbial community establishment, across an mixture gradient increasing in serpentine characteristics, (2) determine how the quantity of organic matter influences both plant and microbial communities within our technosols, and (3) examine how plant colonization and microbial activity of our technosols compare to early successional reference environments. Our study is composed of two chapters, each in manuscript format. Chapter 2 focuses on how technosols containing diamond mine waste influence the establishment of native vegetation. Specifically, which mixture(s) of local mineral mine waste substrates promoted the best conditions for plant establishment. Chapter 3 focuses on how technosols containing diamond mine waste influence microbial activity. Both chapters discuss essential components to consider during mine site rehabilitation using mineral waste substrates. In the natural environment, plant and microbial communities influence each other, and together are an essential biological component for creating self-sustaining environments (Batten *et al.*, 2006; Hobbs *et al.*, 2007; van der Heijden *et al.*, 2008), one of the main goals of rehabilitation.

Chapters 2 and 3 are written in manuscript form. For chapters 2 and 3 I assisted in planning the experiment, and was fully involved in the construction of our test plots. I also worked with Dr. Daniel Campbell to determine which parameters to focus on in each study. For both chapters I was also in charge of sampling, data analysis, and writing. Dr. Daniel Campbell was the main intelligence behind the experimental design planning, was involved in brainstorming statistical analyses, and involved in editing. Given my role in these manuscripts I am submitting them in the form of my Master of Science thesis.

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CHAPTER 2: Testing technosols for rehabilitation of diamond mine waste in a subarctic region

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Abstract

Mining and site rehabilitation in subarctic environments can be challenging largely due to the remote location of these developments. Rehabilitation is therefore often limited to using local mine waste materials to manufacture successful technosols. This study focused on how mines in northern regions, specifically the isolated De Beers Victor Diamond Mine, can use local mine waste substrates to manufacture successful technosols. Our objectives were to, (1) determine which mixture(s) of mineral substrates promotes the best conditions for early plant colonization in regards to early physical and chemical development, along a gradient of serpentine conditions; (2) determine how the quantity of peat influences vegetation establishment, and (3) examine the performance of our mixtures in comparison to other early successional reference environments. We constructed a 4 x 2 factorial experiment on two areas of mine waste at the De Beers Victor Mine, the waste rock dumpsite, and a processed kimberlite storage facility. Three test blocks were constructed on each site comprising a total of 48 5 m x 5 m experimental plots with four mineral substrate mixtures, and two levels of peat application (20% and 40% peat). Mixtures consisted of various combinations of coarse and fine processed kimberlite, a silty loam marine overburden, and 20% or 40% peat, creating an ultramafic, serpentine technosol gradient. We fertilized each plot at a rate of 12.5 g m^{-2} (NPK 8-32-16), inoculated them with a microbial 'tea' mixture, and seeded them with a variety of native vegetation. We then examined various early physical, chemical and biological developments of the mineral mixtures, mine waste controls, an existing peat-overburden cover mix at the Victor Mine, and a natural early succession environment. Our results suggest that non-serpentine mixtures (100% overburden-peat) promoted slightly better physical and chemical conditions, reflected in vegetation establishment, than serpentine mixtures containing more processed kimberlite. However, physical and chemical differences between our mineral mixtures were minor. In this study we were unable to achieve a great enough difference in percent peat applications, therefore were unable determine how the quantity of peat influenced vegetation establishment. In comparison to mine waste controls, the experimental mixtures had better conditions for vegetation establishment. The experimental mixtures shared similar vegetation cover and plant species richness to that of an existing peat-mineral soil cover. The experimental mixtures also shared similarities with a natural early successional environment. Early similarities noted may indicate the experimental mixtures' potential for success during rehabilitation. This research will provide the De Beers Victor Mine with suggestions for rehabilitation, information on potential challenges incorporating processed kimberlite into technosols, be useful for other northern developments, and will help develop protocols for mine waste rehabilitation in subarctic regions.

Key words: anthroposol, serpentine soil, ultramafic substrate, processed kimberlite, primary succession, mine reclamation, ecological restoration

Abbreviations

HBL: Hudson Bay Lowland

WR: waste rock dumpsite

PK Cell: processed kimberlite storage facility

OB: silty loam overburden

CPK: coarse processed kimberlite

FPK: fine processed kimberlite

Introduction

Mining developments remove, bury or disturb existing soils. In return, the mines produce waste substrates, including tailings, waste rock and overburden deposits, often with poor structure, a lack of organic matter, nutrient deficiencies and sometimes with elevated metal concentrations, all of which provide unfavorable conditions for plant establishment (Johnson & Van Cleve, 1976; Bradshaw, 1996; Wong, 2003; Hobbs *et al.*, 2007). If left alone, these mining wastes slowly colonize through the process of primary succession (Walker & Del Moral, 2009; Yoshitake *et al.*, 2016). Although primary succession is naturally a slow process (Forbes & Jefferies, 1999), rehabilitation managers can emulate and speed up this process through addressing limitations and adding a biological component (Bradshaw & Chadwick, 1980; Drozdowski *et al.*, 2012; Brown & Naeth, 2014). In some cases, existing soils from nearby sites can be salvaged to rehabilitate the damaged lands (Rowland *et al.*, 2009; Turcotte *et al.*, 2009; Naeth & Wilkinson, 2014). In other cases, new soils, termed technosols or anthroposols, must be created (Chesworth, 2007; Naeth *et al.*, 2012). Physical, chemical, and biological conditions of these new substrates must be addressed to control erosion, restore the soil moisture and thermal regimes, sustain vegetation and increase biomass production, re-establish ecosystem function and improve aesthetic appeal (Johnson & Van Cleve, 1976; Bradshaw & Chadwick, 1980; Bradshaw, 1996; Drozdowski *et al.*, 2012; Brown & Naeth, 2014).

Many challenges accompany rehabilitation in subarctic regions, such as short cool summers and long cold winters (Riley, 2003; Abraham & Keddy, 2005), challenging micro-climates producing frost heave and needle-ice formation (Groeneveld & Rochefort, 2002; French, 2007) and natural deficiencies in essential plant nutrients (Johnson & Van Cleve, 1976), all of which can inhibit the growth and development of native plant communities (Forbes & Jefferies, 1999). Furthermore, subarctic mining developments are often remote, so reclamation is often restricted to using a limited variety of local materials to manufacture effective technosols.

To make a technosol at a mine site, mineral and organic substrates are mixed together with other soil amendments to improve various physical, chemical and biological properties of the waste deposits (Danielson *et al.*, 1983; Li & Fung, 1998; Naeth & Wilkinson, 2014). However, incorporating local mineral substrates into these mixtures can be problematic because the mineral substrates often have poor physical properties and unfavorable chemical characteristics (Reid & Naeth, 2005a, 2005b; Drozdowski *et al.*, 2012; Naeth & Wilkinson, 2014), with the potential to limit soil formation (Wong, 2003).

Diamonds are mined from ultramafic kimberlite deposits (Webb *et al.*, 2004; Sparks *et al.*, 2006). Ultramafic kimberlite is composed of mostly mafic minerals and low levels of silica (Kruckeberg, 2002). As a result, large quantities of processed kimberlite are produced as tailings. Processed kimberlite provides challenging physical and chemical conditions for plant growth. Coarse processed kimberlite has a sandy texture that limits its ability to hold water and nutrients, and its dark colour can act as a heat absorber, raising surface temperatures and further limiting surface moisture (Drozdowski *et al.*, 2012). Chemically, processed kimberlite is similar to serpentine soils (Reid & Naeth, 2005a; Hobbs *et al.*, 2007), which form from the weathering of ultramafic rock formations (Kruckeberg, 2002; Brady *et al.*, 2005). They are recognized for containing elevated levels of magnesium in relation to calcium, leading to low calcium to magnesium ratios (Whittaker, 1954; Brady *et al.*, 2005). Serpentine soils may have a variety of pH levels, often contain elevated levels of iron, nickel, chromium, cobalt, and are characterized by low bioavailable macronutrients, essential for plant growth (Whittaker, 1954; Brady *et al.*, 2005). Serpentine soils host specialized plant species or unique assemblages, with lower plant productivity (Brady *et al.*, 2005). Plants adapted to serpentine conditions often possess xeromorphic features or have stunted growth (Brady *et al.*, 2005). Studies focused on incorporating processed kimberlite into soil covers found that when physical and nutrient limitations of the processed kimberlite tailings material were addressed, plant growth improved (Reid & Naeth, 2005a, 2005b; Drozdowski *et al.*, 2012; Naeth & Wilkinson, 2014).

Organic matter plays an important role in establishing a functional soil (Larney & Angers, 2012). A variety of organic-rich materials can be used to manufacture technosols that vary in their suitability, availability, and their cost. These are important considerations for remote mining developments, where transportation can be a challenge (Larney & Angers, 2012). Salvaging local organic material must often be considered. In northern Alberta, local peat is incorporated into the technosols manufactured after bitumen mining because of the abundance of peat in peatlands disturbed by the mining footprint (Rowland *et al.*, 2009). Mixing 15 to 50 cm of peat with a mineral overburden has been found to improve water holding capacity, nutrient availability, cation exchange capacity and the availability of organic substrates for microbes, with benefits to plant communities and overall ecosystem function (Danielson *et al.*, 1983; Reid & Naeth, 2005a, 2005b; Rowland *et al.*, 2009; Larney & Angers, 2012).

In this study, we examined how remote diamond mines can use local mine wastes and organic substrates to manufacture technosols for mine site rehabilitation. The objectives of the study were to (1) determine which mixture(s) of mineral substrates promote the most suitable conditions for the establishment of vegetation, along a serpentine gradient; (2) determine how the quantity of peat influences vegetation establishment, and (3) examine the performance of our mixtures in comparison to other early successional reference environments. We focused on early physical and chemical development and early plant colonization on our technosols. It was hypothesized that non-serpentine mixtures and those containing higher amounts of peat would produce more functional soil conditions for successful vegetation establishment.

Materials and Methods

STUDY AREA

The De Beers Victor Diamond Mine is situated in Ontario’s Far North, within the Attawapiskat River watershed of the Hudson Bay Lowland (HBL; 52° 49' 08" N 83° 54' 52" W; 80 m elevation). The HBL is recognized as the third largest wetland in the world, south and west of James Bay and southwest of Hudson Bay, with an area of 373 700 km^2 (Abraham & Keddy, 2005). The HBL consists of Paleozoic limestone, overlain in the area of the mine by marine silty-clay to clayey-silt deposits, glacial till, glacio-fluvial, and glacio-lacustrine deposits, ranging from < 5 m to 30 m in depth, and capped by ~ 2 m of peat (Martini *et al.*, 2006; De Beers Group of Companies & AMEC, 2014). The landscape is very flat and poorly drained, covered ~ 95% by extensive peatlands, both fens and bogs, with discontinuous permafrost (Riley, 2011). Upland environments only account for a small percentage of the HBL terrain (Riley, 2003; Abraham & Keddy, 2005). They are associated with old beach ridges, limestone outcrops, eskers, and paradoxically, with river valleys and river island shorelines (Riley, 2003; Abraham & Keddy, 2005).

The climate is dominated by a humid, high boreal eco-climate, greatly influenced by its proximity to Hudson Bay and James Bay (Riley, 2003; Abraham & Keddy, 2005). The region has short cool summers and long cold winters. The mean annual temperature is -1.3 ^{o}C, the mean temperature in January is -22.3 ^{o}C, whereas the mean temperature in July is 17.2 ^{o}C (Lansdowne House; 52°14' N, 87°53' W; 280 km WSW; 254 m elevation; 1971-2000 normals; Environment Canada, 2016). The mean annual precipitation is 700 mm, over half of which falls during the growing season from June to September. The average effective growing degree-days above 5 ^{o}C in the vicinity of Victor Mine range from 750 to 1000 (Agriculture and Agri-foods Canada, 2014). A total of 457 vascular plant species exist in the Attawapiskat River watershed of the HBL, of which almost half are upland or facultative upland species (Riley, 2003). The small patches of upland environments often support a greater variety of vegetation than the vast surrounding peatlands (Ritchie, 1957).

A series of kimberlite pipes located in the HBL approximately 90 km west of the small community of Attawapiskat launched the Victor Diamond Mine, Ontario's first diamond mine, operated by De Beers Canada. The Victor Mine is an open pit mining development that opened in 2008, with a ~ 11 year mine life. Two adjoining diamond-bearing kimberlite pipes were mined, known as Victor Main and Victor Southwest (Webb *et al.*, 2004). These kimberlite pipes have predominantly archetypal spinel carbonate kimberlite, with olivine grains (31%), juvenile lapilli (31%), and an inter-clast matrix (26%), with lesser proportions of country rock fragments (6%) and mantle-derived xenocrysts (6%) (Webb *et al.*, 2004). Their mineralogy consists of serpentine, olivine grains, dolomite, and calcite clasts with minor constituents of phlogopite, quartz, orthopyroxene, diopside, magnetite/spinel, chlorite, garnet, iron oxythydroxides, ilmenite, and pyrite (Lakefield Research, 2003). The milling process produces two tailings, a coarse processed kimberlite (CPK) and a fine processed kimberlite (FPK). Limited chemicals are used during the kimberlite processing (De Beers Group of Companies & AMEC, 2014); a non-toxic, chemically stable, FeSi reagent powder is used and recovered for re-use.

Approximately 17.4 Mt of silty loam marine overburden and 1.2 Mm^3 of sphagnum-dominated peat were removed to access the kimberlite pipes (De Beers Group of Companies & AMEC, 2014). The mineralogy of the overburden consists of calcite, dolomite and quartz with minor constituents of plagioclase, chlorite, and muscovite (Bergeron & Campbell, unpublished). The acid generating potential of the kimberlite and surrounding limestone is very low, with no potential for acid mine drainage due to the presence of carbonate minerals improving the neutralizing potential and lack of sulfide minerals (De Beers Group of Companies & AMEC, 2014).

Upon closure, mine management plans to rehabilitate the mine site (~ 900 ha), including the mine camp, the roads, tailings facilities, overburden and waste rock piles, into well-drained upland forested hills, which were not present in the conditions prior to disturbance (De Beers Group of Companies & AMEC, 2014). Mine managers plan to use local waste materials, including peat, overburden, and processed kimberlite, to create a soil medium during the reclamation process. Their objectives are to control erosion, ensure physical stability, and accelerate the development of self-sustaining, native vegetation (De Beers Group of Companies & AMEC, 2014).

EXPERIMENTAL DESIGN AND SAMPLING

We constructed a factorial experiment during the summers of 2013 and 2014 on two areas of mine waste at the Victor Mine, a limestone waste rock dump (WR), and a processed kimberlite storage facility (PK Cell), separated by 2.5 km. The WR dump was ~ 1 km^2 in size and the area of the experiment was generally flat with a matrix of fragmented limestone rocks with good drainage and sparse early successional vegetation. The PK Cell was constructed of CPK and the plots were located on a west-facing 3:1 slope. The PK Cell was constructed of coarse processed kimberlite. In 2015, the company placed a cover layer of 40 cm of overburden followed by a 10 cm cover of peat over most of the North and West side slopes of the PK Cell in 2015, except near the experimental blocks, and mixed the top 10 cm to create cover mix of silty marine overburden and peat and it was not seeded. We built three test blocks on each waste site, with 50 to 100 m between blocks. For each block, a bulldozer was used to dig shallow trenches ~ 50 to 80 cm deep by 60 m in length and 7 m in width. We used these shallow trenches to avoid excessive winterkill in the experiment. Plots were built up within the trenches, so they were ~ 50 cm in height, to be flush with the surrounding ground surface (Appendix, Figure A1). Each block (n=6) contained eight experimental plots, measuring 5 m by 5 m in size (n = 48), separated by 4 m. Each block contained one of the eight factorial treatments we created.

To create these factorial treatments, we combined three mineral mine waste substrates and peat to create technosol mixtures, including the marine overburden (OB), CPK, and FPK. The OB was dominated by silt-sized particles, but also contained clay-sized to coarse sand-sized particles, producing a silty-loam texture overall (Denholm & Schut, 1993; Appendix, Figure A2a). The CPK was dominated by the sand fraction, with a coarse sand texture overall, while the FPK was dominated by a fine sand texture, with a loamy-sand texture overall (Denholm & Schut, 1993; Appendix, Figure A2a). The raw peat was dominated by coarse organic fragments > 0.25 mm in size. The raw peat had organic matter content ranging from ~ 20% to > 30%, whereas OB, CPK, and FPK had organic matter contents of < 10%. The mineral substrates were alkaline, ranging from pH 7.8 to 8.3 in the OB to pH 8 to 8.8 in the FPK and CPK; the peat was more acidic with a pH 5.2 (Appendix, Table A1). Raw OB was rich in total calcium, and it also had the greatest electrical conductivity compared to the CPK and FPK, which were rich in total magnesium. Raw peat contained the highest total carbon and nitrogen. Total N, P, and moderate amounts of K were detected in all raw materials (Appendix, Table A1). The ultramafic CPK and FPK had greater total concentrations of Fe, Ni, Mn, Cr, Cu, Co, and Se (Table A1). The carbon to nitrogen (C:N) ratio of the raw peat was the lowest (mean 22:1), followed by OB (123:1), FPK (194:1), and CPK with the greatest C:N ratio (mean 313:1). Total nickel, chromium, and selenium exceeded Canadian Council of Ministers of the Environment (total CCME) industrial soil quality guidelines (Appendix, Table A1) for the mineral mine waste substrates. However, the bioavailability of these heavy metals was low in these materials. Peat also had the highest cation exchange capacity (CEC) with a median of 128 cmol kg^{-1}, followed by FPK, then silty loam OB and CPK with lower CEC < 100 cmol kg^{-1} (Bergeron & Campbell, unpublished).

We first created four mixes using these mineral substrates to create a non-serpentine to serpentine gradient, based on preliminary growth chamber trials: (i) 100% OB; (ii) 50:50 OB:CPK; (iii) 50:25:25 OB:CPK:FPK; and (iv) 25:50:25 OB:CPK:FPK. This created a gradient of 100% OB to a mineral mix of 75% kimberlite. We made the mineral mixes using the bucket of a small tractor, applying alternate scoops of material with a volume of 0.137 $m^{3,}$ and periodically mixing with a rake and disk harrow. Then, two levels of peat were mixed in using the disk harrow (either 20% or 40%) for a total of 4 x 2 factorial treatments.

During late August 2014, we created a ‘litter tea’ for microbial inoculation, separately for each block, by collecting ~ 4.5 L of forest litter and humus from under spruce trees (*Picea glauca* or *P. mariana*) and under buffaloberry (*Shepherdia canadensis*) and alder (*Alnus crispa* var *viridis* or *A. incana* var *rugosa*) from the Attawapiskat River valley. To this we added 1 to 4 crushed nodules from *Vicia americana* and *Alnus* species. We steeped the litter and crushed nodules for ~ 12 hours in 54 L of room temperature tap water, mixing occasionally. This mixture was then strained through a 2 mm mesh sieve. We watered each plot with 6 L of this tea using a watering can. We fertilized the soil mixtures at a rate of 12.5 g m^{-2} with a dry blend fertilizer (8-32-16 NPK). We then seeded each mixture with a variety of native nitrogen-fixing shrubs, nitrogen-fixing herbs, grasses, forbs, and shrub seeds that were collected locally during 2014 (Table 1).

We compared our experimental mixtures with four other reference substrates (Appendix, Figure A3), (i) the surrounding limestone waste rock dump (WR dump); (ii) a surrounding cover mix of OB and peat on the PK Cell; (iii) a cover mixture of OB and peat on the South OB stockpile; (iv) a primary successional site in the floodplain of the Attawapiskat River. The WR dump and the PK Cell cover mix sites (described above) surrounded the experiment so these references acted as partial controls. The South OB stockpile is a ~ 7 ha area of marine silty loam OB with a 30 to 50 cm thick 1:1 overlying cover mix of OB and peat. This cover mix was placed by the company in 2011 and was not seeded, but vegetation is slowly recolonizing naturally. These peat and mineral mix covers are a common practice in northern mine reclamation (Danielson *et al.*, 1983; Moskal *et al.*, 2001; McMillan *et al.*, 2007; Rowland *et al.*, 2009; Turcotte *et al.*, 2009), so the South OB site allowed an early successional comparison with our experiment. The river floodplain has a similar overburden substrate and is well drained in the growing season, but is gouged by winter ice on annual to decadal scales, so has no observable soil horizon development. It has diverse early successional vegetation so represents a diverse reference endpoint for this experiment. Finally we sampled the East OB site, an ~ 85 ha area of marine silty loam OB ~ 10 m above the surrounding landscape with no peat amendments and sparse natural plant colonization. It has not been reclaimed, so it allowed a contrast with OB treatments in the experiment. On each reference location, we sampled ten locations approximately 20 to 50 m apart. We also sampled an additional 5 m x 5 m plot on the South OB stockpile and at the river site.

We sampled the experiment and reference sites primarily during the 2015 growing season, with supplemental sampling for microclimatic and vegetation in the

2016-growing season. For several parameters, as detailed below, we collected five subsamples from the 5 m x 5 m experimental and reference plots, one from the middle and four within ~ 75 cm of each corner. Additionally, we collected subsamples from three sides of some experimental plots for several parameters since they evidently had different conditions, creating a total of nine subsamples of sides per plot. Limitations to our sampling included a washout of one complete plot on the PK Cell before sampling began in 2015, partial washouts of seven plots on the PK Cell before sampling began in 2015, a complete washout of one plot on the PK Cell before sampling began in 2016, a lost block on the WR dump before the 2016 season, and the loss of the East OB stockpile before sampling began in 2016.

For loss-on-ignition (LOI), we sampled four raw substrates from each waste site (n = 4 each), and all the reference sites (n = 10 each). We also sampled one experimental block per waste site with subsamples on the tops and the sides of each plot. We placed 5 g of dried material of each subsample in 10 ml crucibles, then placed them in a muffle furnace at 650 ^{o}C for 7 hours and then reweighed them. LOI subsamples within experimental plots were averaged prior to statistical analyses.

For bulk density, we took subsamples on the tops and sides of all experimental plots using a 7.6 cm diameter by 5 cm deep hole saw. We also took a total of ten samples from each reference site. We weighed the samples after they dried at 105 ^{o}C for 24 hours.

We measured root penetrability resistance at five subsample locations of experimental plots within one block per waste site. We also sampled each reference site location ten times. A 1 m Eijkelkamp® hand penetrometer (model 0.601.SA), with 2 cm^2 and 3.3 cm^2 cones was used at 5 cm increments to a depth of 25 cm.

We measured surface roughness as a measure of surface changes from frost movement using the method of Saleh (1993). We took two measurements per plot in one block at each waste site in early June of 2015 and 2016. Ten measurements were taken at each reference site in 2015, but in 2016 only one site at the South OB stockpile and river was examined. To do this we laid a bicycle chain across 1 m of the soil surface following the grooves of the surface closely. A measurement of the length of the bike chain when pulled taught was recorded and used to calculate the surface roughness index.

Volumetric water content (VWC) was measured using a Delta-T Devices W.E.T.® sensor kit. Data was collected every two weeks from June to August 2015 at all subsample locations on the tops and sides of experimental plots and at the river and South OB stockpile reference plots. Surface water content changes were monitored for the experimental mixtures in one block at each waste site 24, 48 and 72 hours after a soaking rainfall event (July 26 to 28, 2015). To calibrate the sensor, we simultaneously collected surface samples by hand using a 7.6 cm diameter by 5 cm deep hole saw. Samples were bagged and weighed within 6 hours, then dried at 105 ^{o}C for 24 hours, and reweighed to obtain their dry weight. VWC was then calculated to calibrate the W.E.T. measurements ($y = 0.71x + 0.03$; $r = 0.672$; $n = 336$).

We installed various data loggers at the PK Cell, WR dump, South OB stockpile and along the river to measure temperature, soil water content, and electrical conductivity. We first measured aboveground temperature 1.5 m above the soil surface at each site using HOBO® Pro V2 (model U23-002) Temperature and Relative Humidity data loggers in hand-made radiation shields. We also followed a depth gradient for capture of soil water content, temperature and conductivity within the experimental soil mixtures containing 100% OB and 50:50 OB:CPK with 20% at each waste site, at the South OB stockpile, and along the river. Measurements were taken at -3 cm using Decagon® GS3 sensors and at -5 cm, -10 cm, -15 cm and -20 cm depths using Decagon® 5TE sensors, with measurements logged every half hour from July 2015 to August 2016 using Decagon® Em50 data loggers, except for three weeks in August 2015 along the river. We calculated growing degree-days (GDD), with a base temperature of 5 ^{o}C, using daily aboveground air temperature data from July 4 to October 31 2015 and April 1 to August 23 2016, and -3cm below the soil surface from July 22 to October 31 2015, and April 1 to August 23 2016.

For chemical analyses, we sampled all subsample points on the tops of each experimental plot and bulked them within each plot. We also took five samples from each reference site. We dried each soil sample at 105 ^{o}C for 24 hours and sieved them through a 2 mm mesh screen. Using part of each sample, we prepared lithium nitrate extractions as a measure of bioavailable concentrations (Abedin *et al.*, 2012), which were analyzed using ICP-MS by Testmark Laboratories, in Sudbury, ON. Samples of raw mineral mine waste substrates, peat, South OB stockpile, and river soil were analyzed for nitrogen and sulphur content using an Elementar Vario EL Cube® CNS analyzer at LUCAS Services in Thunder Bay, ON. Due to the high concentration of calcium from $CaCO_3$ in the raw materials, total organic carbon content was also determined using LOI values using a conversion (Total C = (0.443 x LOI) - 2.77) suggested by Wright *et al.* (2008).

We measured the soil pH and electrical conductivity (EC; as an estimation of soil salinity) in early June and again in late August 2015 for the subsamples on the soil surface on the tops of the experimental mixtures, and at -20 cm depth for one block on each waste site. We measured soil pH and electrical conductivity for subsamples on the sides of the experimental mixtures only in June 2015. For the remaining two blocks on each waste site, subsamples were bulked within plots before measurement. We also measured pH and conductivity at the soil surface and at -20 cm at each reference site ten times. For the analyses, we mixed 10 g of fresh soil with 40 mL of de-ionized water, stirred by hand for five minutes and used a Fisher Scientific Accumet AB15 Basic® pH meter and a Thermo Scientific Orion 3 Star® electrical conductivity meter.

We examined vegetation establishment in late August, of 2015 and 2016. We randomly tossed quadrats measuring 25 cm x 25 cm at random 15 times on the top of each experiment plot and 9 times on their sides. Vegetation establishment on each plot side was measured for one block on each waste site in 2015 and for all blocks in 2016. In addition, we sampled each reference site ten times, each with 15 quadrat tosses, except at the river where we only performed eight random quadrat tosses. We noted presence of plant species and total plant cover using the following a modified Braun-Blanquet system (1: <1%, 2: 2-5%, 3: 6-20%, 4: 21-50%, 5: >50%). We examined aboveground biomass on each plot, at the South OB stockpile plot, and at the river plot by clipping all plant material at the soil surface in a 50 cm by 50 cm quadrat, drying it at 60 ^{o}C for 48 hours, then weighing their mass.

STATISTICAL ANALYSES

We first analyzed only the experimental mixtures, usually as a 2 x 3 x 2 x 4 (waste site x block x peat x mineral) factorial analyses of variance for separate dependent variables, including loss-on-ignition, bulk density, surface roughness, soil water content, pH, electrical conductivity, total carbon, nitrogen and sulphur, total plant cover, above ground biomass and plant species richness. Blocks and waste sites were considered as random factors and we did not include their interaction terms in the analyses. We employed a repeated measures analysis to examine for changes in surface pH and electrical conductivity between June and August 2015. The pH measurements were averaged prior to analyses using H^+ concentrations. We used split-plot analyses to examine root penetrability, with depths as subplots. We used Tukey tests for post-hoc analyses. As a second step, we compared the tops of the entire mixtures experiment to the reference sites with the exception of the East OB stockpile, again using analyses of variance. We compared the results from our 100 % OB-peat mixtures to the East OB stockpile separately using the same analyses. We verified assumptions of all analyses graphically and transformed the data logarithmically, when necessary. SPSS®, version 21, was used to conduct all univariate analyses.

To test for multivariate differences in plant assemblages, species present in < 2% of plots were removed. We used a square-root transformation on the data and then calculated the matrix distances using the Bray-Curtis dissimilarity measure. To test for multivariate differences in soil nutrients, we used the square-root transformation on the data and then calculated the matrix distances using the Euclidian distance measure. We then performed multivariate analyses of variance, with 9999 permutations, using PERMANOVA+ in PRIMER®, version 7. We used pairwise PERMANOVA tests, followed by SIMPER analyses, to evaluate significant differences. For all analyses, we set a Type I error value of 5%, and considered those within +10%.

Results

MIXTURES

The organic matter content of the mixtures ranged from < 10% to ~ 20% ($P = 0.420$; Appendix, Table A2), and contrary to our intent, we found no difference in LOI between the 20% and 40% peat applications ($P = 0.885$; Appendix, Table A2). This lack of difference in the LOI between the peat amendment treatments was also reflected in a lack of significant differences between peat treatments in bulk density, root penetrability, surface roughness, volumetric water content, pH and conductivity, and even vegetation cover, composition, and aboveground biomass.

The bulk density differed between our mineral mixes ($P = 0.024$; Appendix, Table A3; Figure 1a), with the greatest difference between the mixtures containing 100% OB and those consisting of 25:50:25 OB:CPK:FPK, with the latter having greater bulk density. When we compared the tops of the plots against the sides of the plots, the bulk densities of the tops of the PK Cell plots were lower than the sides, but this was reversed at the WR dump, with the side samples having lower bulk densities than the tops. Despite these slight differences in bulk density, we found no difference in root penetrability resistance with depth among the different mineral mixtures in our experimental plots ($P = 0.739$; Appendix, Table A4), with a mean resistance across all the mineral mixtures at all depths of 125.6 N cm^{-2}.

The surface roughness of our mineral mixtures was initially measured in June 2015 ($P = 0.030$; Appendix, Table A5; Figure 1b); the 50:25:25 OB:CPK:FPK had slightly lower surface roughness index (SRI) values than the other three mineral mixtures. The mixtures constructed on the WR dump also had slightly higher SRI values than on the PK Cell cover mix ($P = 0.007$; Appendix, Table A5). These differences between mineral mixtures and between waste sites disappeared by June 2016 (P = 0.614; Appendix, Table A5).

The volumetric water content (VWC) of the mineral mixtures differed. VWC was highest in the mixtures containing 100% OB and lowest in those with higher amounts of CPK (P < 0.001; Appendix, Table A6; Figure 1c). Mixtures on the WR dump had higher VWC measurements than those on the PK Cell with means of 0.162 m m^{-3}, and 0.117 m m^{-3} respectively (P < 0.001; Appendix, Table A6). Over a 72-hour period after a rainfall event, an interaction was found between the mineral mixtures and sampling time (P = 0.027; Appendix, Table A7; Figure 2). The mixtures containing 100% OB had the highest and most consistent VWC after 24, 48 and 72 hours, whereas the 50:50 OB:CPK and 25:50:25 OB:CPK:FPK mixtures decreased in VWC. When we examined changes with depth, the 50:50 OB:CPK mineral mixtures experienced more variation in soil water content and temperature at all depths during the summer of 2015 (Figure 3) as well as over the entire year (Appendix, Table A8).

All of the mineral mixtures were quite alkaline, between pH 8 to 9, with mixtures having more kimberlite being slightly more alkaline than those containing 100% OB (P = 0.023; Appendix, Table A9; Figure 1d). Surface pH decreased slightly between June and August (mean pH 8.71 and 8.47 respectively; P = 0.001; Appendix, Table A9). We observed an interaction between sample depth and time of year sampled ($F_{1, 45}$ = 20.15; P < 0.001). In this interaction soil pH at 20 cm depth was greater than surface pH during June (mean pH 9.16 versus pH 8.70) and August (mean pH 8.63 versus pH 8.43) 2015. When we compared the tops and sides of the experimental mixtures in June and August

2015, we found the tops of the mixtures had higher pH than samples from the side slopes (mean pH 8.70 versus pH 8.17, respectively; $F_{1, 22} = 59.35$; $P < 0.001$).

The 100% OB and 50:25:25 OB:CPK:FPK mixtures had the highest electrical conductivities (EC) in June and August 2015 ($P = 0.015$; Appendix, Table A10; Figure 1e). The EC of the 25:50:25 OB:CPK:FPK and 50:50 OB:CPK mixtures increased from June to August, whereas the 100% OB mixtures EC decreased from June to August ($P = 0.003$; Appendix, Table A12). Soil EC at a depth of 20 cm was not significantly different than at the surface in June and August 2015 ($F_{1, 116} = 1.93$; $P = 0.168$). The EC of the sides of the plots were greater and more variable than those from the tops of the experimental mixtures ($F_{1, 100} = 123.8$; $P < 0.001$), with mean measurements of 199 µs cm^{-1} and 92 µs cm^{-1}, respectively. The experimental mixtures on the WR dump also had greater EC than those constructed on the PK Cell, with mean values of 133 µs cm^{-1} and 91 µs cm^{-1} respectively ($P = 0.041$; Appendix, Table A10).

The concentration of bioavailable elements differed between the mineral mixtures ($P < 0.001$; Appendix, Table A11; Appendix, Table A12; Figure 4). The greatest difference was between the 100% OB mixture and all other mixtures. The main elements responsible for the dissimilarity between all mineral mixtures were calcium and magnesium; a clear calcium to magnesium ratio (Ca:Mg) gradient was observed across the mineral mixtures, with the 100% OB mixture having almost twice as great Ca:Mg ratios than the 25:50:25 OB:CPK:FPK mixture (Figure 1f).

Bioavailable elements also contributed to a minor difference between peat applications rates ($P = 0.007$; Appendix, Table A11; Appendix, Table A12). Mixtures containing 40% peat had slightly more calcium, magnesium, and potassium, and slightly less sodium than the mixtures containing 20% peat.

The concentration of bioavailable elements contributed to minor differences between waste sites ($P < 0.001$; Appendix, Table A11). Mixtures constructed on the PK Cell contained slightly more calcium and magnesium, and less sodium and potassium than those constructed on the WR dump. There were also minor differences in the bioavailable elements of the mixtures between 2014 and 2015 ($P < 0.001$; Appendix, Table A11). During 2015 there was slightly more calcium, and slightly less magnesium and sodium than in 2014.

The mineral mixtures containing 100% OB produced higher total plant cover in 2015 than those containing 50:50 OB:CPK and 25:50:25 OB:CPK:FPK ($P = 0.001$; Appendix, Table A13; Figure 5a). In 2016, the 100% OB mixtures again had the greatest total plant cover, while the other mixtures were statistically similar ($P < 0.001$; Appendix, Table A13; Figure 5b). In 2015 and 2016 the tops of the test plots had higher total plant cover scores than the side slopes ($P = 0.029$, $P = 0.050$ in 2015 and 2016 respectively; Appendix, Table A13). Plots on the WR site also had higher total plant cover scores than the plots constructed on the PK Cell during 2015 ($P < 0.001$).

Mineral mixtures did not affect aboveground biomass in August 2015 ($P = 0.112$ and $P = 0.454$ respectively; Appendix, Table A14) and August 2016 ($P = 0.258$, $P = 0.599$ respectively; Appendix, Table A14). Aboveground biomass was greater on the mixtures constructed on the WR dump than the PK Cell in August 2015 ($P = 0.001$; Appendix, Table A14), but not in 2016. Mean aboveground biomass increased slightly between 2015 and 2016 (mean $3.1e^{-6}$ g m^{-2} versus mean $4.8e^{-6}$ g m^{-2}, respectively).

Plant species richness in 2015 and 2016 did not differ between our mineral mixtures (*P* = 0.421 and *P* = 0.254, respectively; Appendix, Table A15). In 2015 our mixtures had a mean plant species richness of ~ 10, whereas in 2016 they had a mean richness of ~ 8 species. During 2015 and 2016, plant species richness was greater on the tops of our experimental mixtures than the side slopes (*P* < 0.001 and *P* < 0.001, respectively; Appendix, Table A15). During 2015 and 2016, the plots constructed on the WR dump also had greater species richness than the plots constructed on the PK Cell (~ 11 and 9, respectively; *P* = 0.015 and *P* = 0.001, respectively; Appendix, Table A15).

Plant species assemblages differed between the tops of our mineral mixtures in 2015 and 2016 (Pseudo-$F_{3, 37}$ = 1.67; *P* = 0.022). The greatest difference in plant species assemblages was first between the mineral mixtures containing 100% OB and 25:50:25 OB:CPK:FPK (t = 1.655; *P* = 0.008) and second between the mineral mixtures containing 100% OB in comparison to 50:50 OB:CPK (t = 1.477; *P* = 0.031). The 100% OB mixtures contained more *Equisetum arvense* and *Polygonum fowleri* (volunteer species), and *Agrostis scabra* (sown species), whereas the 25:50:25 OB:CPK:FPK and 50:50 OB:CPK mixtures contained more *Erysimum cheiranthoides*. Out of the plant species we sowed on these plots *Beckmannia syzigachne, Poa palustris, Agrostis scabra, Epilobium angustifolium, Achillea millefolium* and *Rubus idaeus* was observed on all mixtures, but were generally more common on the 100% OB mixtures. Plant species assemblages also changed in general from 2015 to 2016 (Pseudo-$F_{1, 31}$ = 8.798; *P* < 0.001). In August 2015, we saw more *P. folweri,* and *E. cheiranthoides*, while in 2016 we saw more *A. scabra*, and *E. arvense*. During 2016, we also saw more *B. syzigachne, A. millefolium, P. palustris, E. angustifolium*, and the emergence of *R. idaeus*.

There was a difference in plant species assemblages when the tops of the mixtures were compared to the sides in 2015 and 2016 (Pseudo-$F_{1, 121}$ = 21.225; $P < 0.001$). *P. palustris*, *E. cheiranthoides*, *A. millefolium*, *Artemesia tilesii*, *A. scabra*, and *E. arvense* were more abundant on the tops of the experimental mixtures than on the sides. The experimental mixture tops also had more *B. syzigachne*, *E. angustifolium*, and *R. ideaus*.

During 2015 and 2016, plant species assemblages also differed between the PK Cell and WR dump (Psuedo-$F_{1, 37}$ = 29.420; $P < 0.001$). The main plant species responsible for the dissimilarity between sites was *P. fowleri*, *E. cheiranthoides*, *A. scabra*, and *A. tilesii*. These plant species were more abundant on the tops of the experimental mixtures constructed on the WR dump. The mixtures on the WR dump also had more *P. palustris*, *A. millefolium*, *B. syzigachne*, and *E. angustifolium*.

EXPERIMENT VS REFERENCE SITES

The WR dump and the PK Cell around the experimental plots had silty sand to loamy sand textures (Denholm & Schut, 1993; Appendix, Figure A2b). The East OB stockpile had a silty loam texture whereas the South OB stockpile and the Attawapiskat river floodplain were both loamy very fine sand to silty sand in texture (Denholm & Schut, 1993; Appendix, Figure A2b).

The experimental mixtures had more organic matter than the surrounding WR dump (~ 3%), but less than the surrounding PK Cell cover mix (~ 24%), that had also received a peat amendment. The experimental mixtures had similar organic matter contents to the South OB stockpile mix (~ 11%) and more than the river soil (~ 7%; *P* <

0.001; Appendix, Table A16a). Our 100% OB-peat mixtures also had more organic matter than the East OB stockpile (P = 0.001; Appendix, Table A16b).

Bulk density in our experiment (mean 0.66 g cm^{-3}) was significantly lower than in the surrounding WR dump (mean 0.93 g cm^{-3}), but higher than the surrounding PK Cell. (mean 0.44 g cm^{-3}; $P < 0.001$; Appendix, Table A17a). Our experimental mixtures had similar bulk densities to both the South OB stockpile and river soil (Figure 6a). Our 100% OB-peat mixtures certainly had lower bulk density than the East OB stockpile (mean 1.05 g cm^{-3}; $P < 0.001$; Appendix, Table A17b).

Following these differences in bulk density, we found differences in root penetrability resistance measurements ($P < 0.001$; Appendix, Table A18a). The WR dump surrounding our experimental plots had greater root penetrability resistance measurements (mean 391 N cm^{-2}), while the surrounding PK Cell cover mix had less resistance (mean 98 N cm^{-2}). The South OB stockpile had slightly less resistance than our mixtures, while the river soil was similar to our mixtures (Figure 6b). Our 100% OB-peat mixtures experiment had similar resistance in as in the East OB stockpile (P = 0.226; Appendix, Table A18b).

The surface roughness of our experimental mixtures was similar to all reference sites examined in 2015 and 2016; with the exception of lower SRI on the PK Cell cover mix in 2015 ($P < 0.001$; Appendix, Tables A19a and A19b; Figure 6c).

The WR dump surrounding the experiment had comparable VWC from June to August 2015, while the PK Cell cover mix, the South OB stockpile and the river had greater soil VWC (means 0.251 $m^3 m^{-3}$, 0.299 $m^3 m^{-3}$ and 0.280 $m^3 m^{-3}$, respectively; P =

0.001; Appendix, Table A20a). The East OB stockpile had similar VWC measurements to our 100% OB-peat mixtures ($P = 0.161$; Appendix, Table A20b).

When changes in soil water content and temperature with depth were examined, the surface of our experimental mixtures had lower soil water contents than the surface of the river soil, and lower water contents at all depths compared to the South OB stockpile (Figure 7). Our mixtures also experienced higher maximum and lower minimum temperatures at all depths than the South OB stockpile and river soil (Appendix, Table A8).

The WR dump surrounding the experimental plots had comparable pH measurements, while the PK Cell cover mix, South OB stockpile, and river soil all were lower with means of approximately pH 8.4 to 8.2 ($P < 0.001$; Appendix, Table A21a). An interaction was found between site pH and time of year sampled, where our mixtures, the WR dump, PK Cell cover mix, and South OB stockpile decreased slightly from June to August 2015 from mean pH 8.7 to 8.5 for the WR dump and mean pH 8.4 to 8 for the PK Cell cover mix and South OB stockpile, while the river soil increased slightly from pH 8.1 to 8.2 ($P < 0.001$; Appendix, Table A21a). Compared to our 100% OB-peat mixtures, the East OB stockpile was more alkaline (mean pH 8.9; $P < 0.001$; Appendix, Table A21b).

Our experimental mixtures had similar ECs to the surrounding WR dump, PK Cell cover mix, and river soil, while having lower ECs than the South OB stockpile ($P < 0.001$; Appendix, Table A22a; Figure 6d). In addition, our mixtures behaved similarly to the river soil in changes in electrical conductivity to a depth of 20 cm (Figure 7).

Compared to our 100% OB-peat mixtures, the East OB was more saline (mean 188 μs cm^{-1}; $P < 0.001$; Appendix, Table A22b), but did not differ with depth ($P = 0.222$).

The C:N ratios of the river floodplain soil and South OB stockpile were both 25:1. The C:N ratio of the river floodplain soil was much lower than the raw mineral mine waste substrates used to construct our experimental mixtures. The bioavailable nutrients of our experimental mixtures differed from the reference sites in 2015, despite a very wide dispersion ($\text{Pseudo-F}_{4,\,113} = 26.209$; $P < 0.001$; Table A23; Figure 8). Our mixtures contained more bioavailable Ca and Mg than the surrounding WR dump and more Ca than the surrounding PK cell. The experiment also had more Mg and K and less Ca than the river soil, and less Ca and S than the South OB stockpile. The river soil also contained more elevated levels of bioavailable heavy metals Fe, Ni, Cu, and Co than our experimental mixtures (Table A23).

Compared to our 100% OB-peat mixtures, the East OB stockpile contained more bioavailable Na, S, and less Ca than our mixtures ($\text{Pseudo-F}_{2,\,14} = 6.073$; $P < 0.001$; Table A23). In addition, the Ca:Mg ratios of the WR dump and PK Cell cover mix, South OB stockpile, and river all had higher Ca:Mg ratios than the surrounding experimental plots with mean ratios of 7:1, 13:1, 13:1, and 12:1, respectively. The Ca:Mg ratios of our 100% OB-peat mixtures was greater than that of the East OB stockpile with a mean ratio of 5:1.

The WR dump and PK Cell cover mix surrounding the experimental plots had far less total plant cover, and species richness in both 2015 and 2016 (mean plant cover scores < 2, Appendix, Table A24a; mean species richness < 6 species, Appendix, Table A25a). Our experimental mixtures had similar total plant cover scores (Appendix, Table

A24a; Figure 6e), species richness (Appendix, Table A25a; Figure 6f), and biomass (Appendix, Table A26) to the South OB stockpile in both 2015 and 2016. The experimental mixtures had far less total plant cover (Appendix, Table A24a; Figure 6e), similar biomass (Appendix, Table A26) and far less richness (Table A25a; Figure 6f) than at the river floodplain. The East OB stockpile, which had not been seeded, had greater total plant cover scores (mean score of ~ 4; $P = 0.002$; Appendix, Table A24b) and less richness than our 100% OB-peat mixes (mean richness < 9 species; $P < 0.001$; Appendix, Table A25b).

Plant species assemblages were significantly different between our experimental mixtures and all reference sites in both 2015 and 2016 (Pseudo-$F_{4, 88} = 33.224$; $P < 0.001$; Figure 9). *P. palustris*, *E. cheiranthoides*, *A. millefolium*, and *A. scabra*, were more abundant on our mixtures than at the WR dump and PK Cell cover mix controls. Several plant species present in our seed mixes were not present on the WR dump and PK Cell cover mix, including *B. syzigachne*, *A. millefolium* and *R. idaeus*. Our mixtures contained more *P. palustris*, *A. millefolium*, *E. angustifolium*, *B. syzigachne*, and *R. idaeus* than the South OB stockpile. The river floodplain was more diverse with a greater variety of grass species, and herbaceous and woody species than our experimental mixtures (Appendix, Table A27). The 100% OB-peat mixtures contained more *P. palustris* and *E. cheiranthoides* than the East OB stockpile (Pseudo-$F_{2, 19} = 5.728$; $P < 0.001$). Several plant species present on the 100% OB-peat mixtures were not present on the East OB stockpile, including *A. millefolium,* and *R. idaeus*. An interaction was found in plant species assemblages on the South OB stockpile and river floodplain in 2015 and 2016 (Pseudo-$F_{4, 71} = 4.266$; $P < 0.001$).

The experimental mixtures, South OB stockpile and Attawapiskat River all had accumulated air temperature GDD's between 800 and 1000 during 2015 and 2016 (Appendix, Table A28). The accumulated GGD's for below the soil surface was more variable in 2015 and 2016, ranging from ~ 700 to 1200 (Appendix, Table A29).

Discussion

This field experiment supported our hypothesis suggesting distinctions can be made between the performance and success of technosols (non-serpentine and serpentine) along a gradient of increasing serpentine characteristics. The non-serpentine and serpentine mixtures differed in their early developmental physical and chemical properties largely owing to characteristics of the mineral mine waste substrates. However, most differences noted between the mineral mixtures were only minor. Despite minor differences in many physical and chemical properties, we were able to distinguish a more successful mineral mixture in regards to establishment of vegetation.

Plant establishment and survival are influenced by physical and chemical properties of the growing medium, which are largely defined by climate and local geology (Alberta Environment, 2010). Focusing on the effect serpentine soils have on vegetation allows us to gain a better understanding of the "serpentine syndrome" (Brady *et al.*, 2005), in order to treat serpentine conditions effectively in mine site rehabilitation. It has been suggested that the most limiting factor for plant growth on serpentine soils is their low ratio of calcium to magnesium (Walker, 1954; Brady *et al.*, 2005; Rajakaruna *et al.*, 2009). Natural serpentine soils are suggested to have Ca:Mg ratios generally less than 1 (Loew & May, 1901 as cited by Kazakou *et al.*, 2008). Both calcium and magnesium are essential nutrients for plant growth, but magnesium is not required in large amounts (Bradshaw & Chadwick, 1980). For adequate plant growth the ratio of magnesium to calcium is suggested to be at least greater than or nearly equal (Walker, 1954). In this experiment, a gradient of increasing serpentine characteristics was evident between the mineral mixtures due to elevated levels of magnesium, naturally present in Mg-rich kimberlite.

In this study, we observed a clear difference in total plant cover between the non-serpentine and serpentine mixtures. The most successful mixtures were the non-serpentine mix (100% OB-peat). Of the serpentine mixes, those containing greater quantities of processed kimberlite exhibited limited plant growth. Studies focusing on reclamation of kimberlite mine waste found similar results; plant growth was reduced significantly in mixtures containing more processed kimberlite, owing to both physical and chemical properties (Reid & Naeth, 2005a; Reid & Naeth, 2005b; Drozdowski *et al.*, 2012; Naeth & Wilkinson, 2014). These studies suggest plant growth benefitted from addressing physical and chemical aspects simultaneously, as was explored in this study through the addition of peat and fertilizer.

A common constraint on restoration success is substrate infertility; an added challenge to rehabilitation of disturbed subarctic environments (Hobbs *et al.*, 2007). However, all of our mixtures had Ca:Mg ratios greater than one, suggesting that their ratio was probably not a dominant factor limiting plant growth across our serpentine gradient. The effect of magnesium toxicity is suggested to only influence colonization in serpentine soils with Ca:Mg ratios lower than 1 (Brady *et al.*, 2005). Though the Ca:Mg ratios observed in our serpentine mixtures were not as low as those observed in natural serpentine soils, our serpentine mixtures still experienced lower ratios than our non-serpentine mixtures. In regards to other essential nutrients, both of our non-serpentine and serpentine mixtures were deficient in bioavailable phosphorous, values were generally below detection limits. These results support other research on soils in subarctic regions (Johnson & Van Cleve, 1976) and those with serpentine characteristics (Walker, 1954; Brooks, 1987 as cited by Brady *et al.*, 2005) that are naturally deficient in essential nutrients.

Previous research also suggests that the bioavailability of heavy metals may be a factor impeding plant growth in serpentine soils (Brady *et al.*, 2005), and those containing processed kimberlite (Drozdowski *et al*., 2012; Naeth & Wilkinson, 2014). Levels of heavy metals can be high in serpentine soils and may be absorbed by the plants inhabiting them (Walker, 1954). Plants that develop on serpentine soils often acquire tolerances to their chemical attributes (Shewry & Peterson, 1975; Brady *et al.*, 2005). Contrary to what we expected, the levels of bioavailable heavy metals between our non-serpentine and serpentine mixtures were not significantly different. The main elements causing separation between our mixtures were due to levels of bioavailable calcium and magnesium. Also, despite research suggesting serpentine soils can contain elevated levels of heavy metals such as iron, nickel, chromium, and cobalt (Brady *et al.*, 2005), our serpentine mixtures displayed only moderately high bioavailable levels of some of these heavy metals (Ni, Cu, Mn), while minimal levels of others (Fe, Cr, Co). The moderately high bioavailability of some of these macronutrients may have influenced vegetation establishment in general on all of our mixtures. However, we could suggest the levels of many of these macronutrients may not have influenced the main vegetative differences between our non-serpentine and serpentine mineral mixtures.

The physical characteristics of our non-serpentine (100% OB-peat) and serpentine 50:25:25 OB:CPK:FPK-peat mixes could have provided better conditions for establishing native plant communities, because these mixtures were dominated by finer particle sizes, that could have influenced bulk density, water-holding capacity, and nutrient retention. It was demonstrated in several studies examined by Daddow & Warrington (1983) that root growth is restricted by soil bulk densities above ~ 1.5 g cm^{-3}. Although our finer mixtures did contain lower bulk density measurements, our results suggest that all of our mineral mixtures, serpentine and non-serpentine, were within a suitable bulk density range for plant establishment. This was also reflected in simulated root penetrability by the lack of difference in root penetrability resistance measurements encountered in our non-serpentine and serpentine mixtures. Finer particle sizes could have also improved soil water-holding capacity, which is another crucial component that can influence plant growth. Studies have found incorporating fine textured material into processed kimberlite tailings improves conditions for plant growth (McKendrick, 1997 as cited by Forbes and Jefferies, 1999; Naeth & Wilkinson, 2014).

Though serpentine soils may vary in texture, research suggests their textures can also be associated with lower water-holding capacity and more susceptibility to drought conditions (Whittaker, 1954; Brady *et al.*, 2005; Alexander *et al.*, 2006). Susceptibility to drought was recognized as a great challenge for vegetation in serpentine soils (Chiarucci, 2004). In our experiment, steady drainage and drying conditions were observed after a rainfall event in serpentine mixtures, consisting of coarser processed kimberlite, experienced steady drainage and drying conditions. The coarser, serpentine mixtures in our experiment also experienced more variation in water movement throughout their soil profiles when examined to a depth of 20 cm below the soil surface. Drozdowski *et al*. (2012) found similar results when examining processed kimberlite.

With depth, our coarser serpentine mixtures also experienced greater maximum and minimum range in temperatures throughout their profile than did our non-serpentine mixtures. Not only do serpentine soils range in texture, they also range visibly in colour (Walker, 1954; Alexander *et al.*, 2006). For example, they may have darker, more heat absorbent surfaces due to their ultramafic parent material (Miles & Tainton, 1979 as cited by Reid & Naeth, 2005a; Drozdowski *et al*., 2012). We can speculate that the dark surfaces and coarser texture of our serpentine mixtures increased the higher maximum and lower minimum temperatures at all depths of the soil throughout the year. On one hand, an increase in surface soil temperature could be recognized as a benefit for plant establishment and microbial colonization in subarctic regions. On the other hand, an increase in soil surface temperature may also result in greater drought susceptibility or heat stress.

Needle-ice formation is another factor that could have influenced vegetation establishment on our mixtures. It is recognized as the segregation of ice into thin vertical needles up to 10 cm in length, near the soil surface (Outcalt, 1971 as cited by Lawler, 1993). Needle-ice is commonly observed in subarctic regions (Johnson & Van Cleve, 1976) and is capable of harming vegetation establishment, especially early colonization (Pirez, 1987; Groeneveld & Rochefort, 2002; French, 2007). It is controlled by properties such as soil particle size, water-holding capacity, and temperature due to their role in capillary action (Outcalt, 1971 as cited by Lawler, 1993; Pirez, 1987; Groeneveld & Rochefort, 2002; French, 2007). We can speculate that one of our serpentine mixtures appeared more susceptible to the formation of needle-ice as indicated through surface roughness. In comparison to our very fine textured non-serpentine mixtures, and very coarse serpentine mixtures, the serpentine 50:25:25 OB:CPK:FPK mineral mixes were dominated by ~ 75% silt-sized particles, which is suggested to be optimal texture conditions for needle-ice formation (Pirez, 1987; Groeneveld & Rochefort, 2002; French, 2007). Texture extremes, such as very coarse and very fine soils, can impede needle-ice growth by influencing water movement (Pirez, 1987; Groeneveld & Rochefort, 2002; French, 2007). The surfaces of our experimental mixtures appeared to be rough when observed and measured in 2016, although no differences between mixtures were noted. Although needle-ice formation can be influenced by a variety of factors in the environment (Groeneveld & Rochefort, 2002), above and below ground temperature and texture were the only factors examined in this study used to indicate the potential needle-ice susceptibility of our 50:25:25 OB:CPK:FPK mixtures.

In the vicinity of the Victor Mine there were several days in the year, during the spring and autumn months, where soils were susceptible to needle-ice formation. Below ground temperature loggers revealed establishment of an insulating, but potentially thin, snow cover over the mixtures from late January through March. During this time temperatures recorded below the soil surface were less variable, ranging from approximately -10 ^{o}C to -5 ^{o}C. However, during October to November and April to May, temperatures below the soil surface fluctuated below and above 0 ^{o}C. During this time recorded air temperatures were below 0 ^{o}C whereas temperatures recorded below the soil surface were above 0 ^{o}C, favoring needle-ice formation.

Natural chemical properties of the overburden and processed kimberlite mine waste substrates influenced mixture pH and electrical conductivity, in addition to essential nutrients. The dominant material that makes up both our non-serpentine and serpentine mixtures is recognized as being naturally quite alkaline (Drozdowski *et al.,* 2012; Naeth & Wilkinson, 2014). A strong concern for alkaline soils is that some nutrients can be difficult for plants to absorb or may become unavailable, such as phosphorus (Bradshaw & Chadwick, 1980; Wong, 2003). Drozdowski *et al*. (2012) found similar results and related the lack of nutrient availability to the alkaline influence of processed kimberlite. However, differences between the pH levels of our mixtures, non-serpentine and serpentine, were minor. Therefore, we may not have observed much of a difference in nutrient availability; therefore differences in plant establishment between our mixtures were probably not solely due to pH. Meanwhile, our non-serpentine mixtures were more saline due to the marine origin of the overburden used.

Rehabilitation provides insight into the dynamics of primary succession because it emulates and seeks to speed up this naturally slow process (Walker & Del Moral, 2009). To guide rehabilitation trajectory to a desired structure, plant species that grow commonly in the desired undisturbed ecosystem can be introduced (Turner *et al.,* 1998 as cited by Hobbs *et al.,* 2007). Of the thirteen native upland plant species we seeded, six of those species emerged and their quantities were responsible for the main differences in plant species assemblages across our serpentine gradient; all were more abundant on the non-serpentine mixtures. These results further support the presence of more optimal growing conditions in our non-serpentine mixtures. The emergence of these native plant species on our mixtures also advocates for the success of seeding with native upland plant species on technosols for rehabilitation purposes.

Although above plant ground biomass did not differ between our non-serpentine and serpentine mixtures as expected, biomass did increase from the first year of growth to the second year of growth on all mixtures. These vegetative changes possibly indicate development on our experimental mixtures. Minor natural shifts in plant species richness and composition were noted between first and second year growth. For example, species such as *R. idaeus* only emerged during second year growth. In addition to the sowed species, a variety of other native species emerged. These species were possibly transported by wildlife, wind dispersal, or already existed in the seed bank on the raw material stockpiles from which we constructed our mixtures.

Minor differences were also noted between the tops and sides of our experimental plots. In terms of bulk density, an interaction was found between the side slopes of our mixtures and waste sites. Processed kimberlite washout was common at the PK Cell. Coarse processed kimberlite noticeably collected in the depressions between our experimental mixtures, which could have influenced bulk density and the chemistry of these side slopes. In terms of vegetation establishment, the depressions between our experimental mixtures did not positively influence vegetation establishment on the side slopes as one might expect. These depressions in some ways mimicked “rough and loose” bioengineering techniques generally used to create micro-sites where seeds collect and are sheltered from harsh conditions (Polster, 2011). Vegetation did grow within these depressions; however, no assessments were made. Total plant cover and species richness were lower on the side slopes of our experimental mixtures in comparison to the surfaces, possibly due to seeding only the tops of the experimental mixtures.

Throughout this experiment, physical, chemical and biological properties of our mixtures constructed on the PK Cell and WR dump varied. For instance, mixtures constructed on the WR dump were more compact, had generally smoother surfaces, held more water, contained less calcium and magnesium, were more saline, and had more total plant cover. These differences could be attributed to year of construction. We constructed the mixtures on the WR dump one year prior to those on the PK Cell. Therefore, they sat for an extra year prior to amending the treatments with fertilizer, a microbial inoculant, and native seeds. When examined in the following year, we observed substrate settlement and an extra year of natural growth prior to the start of this experiment. Differences may also be due to the position of soil mixtures relative to the surrounding waste rock landscape. All of our mixtures were situated on a raised waste rock storage facility and lay flat and flush with the WR dump surface. However, in comparison to the surrounding landscape, the mixtures situated on the PK Cell lay on a 3:1 west-facing slope, which could have made them more susceptible to harsh conditions such as wind and water erosion. During fieldwork, high winds and washout of processed kimberlite were noticed in this location. Wind and washout may have been a negative influence on plant growth.

Contrary to our second hypothesis, we found that the quantity of organic matter we applied did not influence vegetation establishment between our mixtures and had little influence on the physical and chemical properties of mixtures across the serpentine gradient we created. Our results indicate that both our 20% and 40% peat applications behaved in similar manners in our experimental mixtures. During the construction phase of the project our peat applications only differed by a few centimeters depth. We had to consider the depth our disk harrow could reach, approximately 20 cm, for adequate mixing of the organic component with the mineral substrate. Therefore 20% and 40% of that depth was used to determine peat applications. Once peat was applied and mixed in, the difference between our 20% and 40% peat applications could have diminished further; tilling could have pushed peat towards the edges and down the sides of the experimental mixtures. The method we used to apply peat to our mixtures was similar to the method that would be used by the Victor Mine during rehabilitation. Our reasoning behind attempting 20% and 40% peat applications was to determine how vegetation establishment was influenced by minimal or commonly used peat applications during rehabilitation.

Adding peat as an organic amendment to mineral mixtures is known as an important component to improve plant growth (Danielson *et al.*, 1983; Reid & Naeth, 2005a, 2005b). Peat is recognized for improving both physical and chemical soil conditions (Danielson *et al.*, 1983; Moskal *et al.*, 2001; Reid & Naeth, 2005a, 2005b; Larney & Angers, 2012; Mackenzie & Naeth, 2010). Although we did not construct control mixtures without the addition of peat, our results suggest that adding peat to the mineral component of our mixtures did improve soil conditions for vegetation establishment. This was evident when they were compared to the mine waste controls. Mine wastes such as the surrounding raw limestone WR dump provide challenging physical and chemical conditions to overcome. Some of these challenges, including coarse texture, lack of organic matter, and low nutrient availability, can be improved when such stressors are addressed (Naeth & Wilkinson, 2014), such as adding an organic amendment. Compared to a mine waste control, our non-serpentine and serpentine soil mixtures exhibited improvements, including greater organic matter content, intermediate bulk densities, root penetration resistance, and adequate water contents with and added organic amendment. These improvements were reflected in improved plant establishment on our experimental mixtures.

To elaborate on the positive influence peat-mineral technosols have on vegetation establishment, we compared our non-serpentine 100% OB-peat mixtures separately to the un-treated, non-serpentine, mineral East OB stockpile. The East OB stockpile can be examined as a "do-nothing" option if mine rehabilitation was to proceed without the addition of peat (Bradshaw, 1996). At the East OB stockpile we observed natural primary succession. Rehabilitation with organic amendments and native plant species can strengthen the primary succession process. In this study we observed a positive vegetation response to our 100% OB-peat mixtures. Compared to the un-treated East OB stockpile, our 100% OB-peat mixtures had beneficial physical and chemical properties for the establishment of vegetation, including an increase in organic matter. The addition of peat lowered the bulk density, reduced alkalinity, and improved nutrient retention capability in our mixtures. Similar results were found by research studying peat-mineral mixtures in the Oil Sands mining developments (Danielson *et al*., 1983). In terms of essential nutrients, the East OB stockpile and our mineral mixtures did not differ greatly and therefore may not have had a large impact on vegetation establishment. The East OB stockpile contained more bioavailable S and Na, probably due to the thickness of the underlying saline OB stockpile material.

The quantity of peat did have a minor influence on the bioavailability of nutrients in our experimental mixtures. Applying more peat could have resulted in greater accumulations of calcium (Reid & Naeth, 2005a), magnesium and potassium. Peat had a higher cation exchange capacity (CEC) in comparison to the raw mine waste mineral substrates used to construct our experimental mixtures (Bergeron & Campbell, in prep.). This indicates that peat has potentially a greater ability to retain nutrients. Reid and Naeth (2005b) found that, without adding an organic matter component such as peat, CEC was lower and essential nutrients were leached from the soil more easily.

Determining the desired outcome of a rehabilitation project is key to evaluating its success. Eventually achieving a sustainable ecosystem structure and function is one of the main goals during the rehabilitation process (Bradshaw, 1996; Hobbs *et al.*, 2007). Following severe disturbances primary succession takes place and as conditions are addressed, is capable of progressing on its own in a suitable trajectory (Bradshaw, 1996; Hobbs *et al.*, 2007). In order to reconstruct successful primary succession, we must gain an understanding of the surrounding natural functioning system (Bradshaw, 1996). Therefore, evaluating technosol performance in comparison to natural sites undergoing primary succession can be indicative of their trajectory for success during rehabilitation (Bradshaw, 1996).

In this study, we compared the performance and success of our experimental mixtures to mine waste controls, other reclamation peat-mineral mixtures, and the local environment to gain a better understanding of how properties of our experimental mixtures influence vegetation establishment. It is often recognized that it is not realistic to achieve exact pre-existing conditions during rehabilitation; therefore, similarities to surrounding reference sites can be used as indicators of their success (Bradshaw, 1996). In our study we found both our non-serpentine and serpentine mixtures exhibited similar characteristics to reference sites in various physical, chemical, and biological aspects. These similarities indicate their potential success for use in mine rehabilitation.

The South OB stockpile peat-mineral mix represents the current technosol being used for rehabilitation at the Victor Mine. Peat-mineral mixtures also are a common practice for rehabilitation of northern mining developments in the Athabasca Oil Sands (Danielson *et al.*, 1983; Moskal *et al.*, 2001; McMillan *et al.*, 2007; Rowland *et al.*, 2009; Turcotte *et al.*, 2009). Our experimental mixtures exhibited similar characteristics to the South OB stockpile peat-mineral rehabilitation mixture at the Victor Mine, with respect to total plant cover, biomass, and species richness. These vegetative similarities are accompanied by similar physical characteristics including organic matter content, bulk density, and surface roughness. Since our mixtures shared similar attributes to a soil cover currently in use, this could suggest our experimental peat-mineral mixtures have the potential to be applied with similar success. However, in some aspects, the South OB stockpile mix displayed potentially more beneficial physical features for plant establishment than our mixtures. These features included easier root penetration, more water availability, lower alkalinity, and more optimal Ca:Mg ratios. One potentially negative aspect of the South OB stockpile mix could be the salinity level, classified as very slightly saline according to the USDA (United States Department of Agriculture). This is possibly the result of deep underlying saline overburden in this stockpile.

Plant community composition was very different on our experimental mixtures when compared to the South OB stockpile, East OB stockpile, and mine waste controls sites. It appears that seeding our experimental mixtures with native upland plant species was effective at introducing desired plant communities. When considering the added beneficial features exhibited in the South OB stockpile mix, it can be postulated that if seeded, native plant species would respond favorably to that mix. Therefore, the knowledge gained from our study could support the success of the South OB stockpile mix for rehabilitation of the Victor Mine, if seeded with native plant species. In terms of a "do-nothing" option, the knowledge gained from our study supports the notion that rehabilitating stockpiles by adding organic amendments followed by sowing native plant species is more beneficial than "doing nothing" to mineral mine waste stockpiles.

Although it is critical to assess our experimental mixtures in comparison to mine waste and other rehabilitation mixtures, it is also important to evaluate where we stand in relation to the natural environment. The desired environment to which the Victor Mine eventually hopes to achieve through rehabilitation is described as a well-drained, upland environment. However, natural, well-developed upland environments amidst the Hudson Bay Lowlands are difficult find and access; therefore they are difficult to use for a natural comparison. Instead, upland environments undergoing primary succession are more commonly observed, and can be found along river shorelines within the Hudson Bay Lowlands. Therefore, for this study, the Attawapiskat River floodplain represented an ideal natural environment to compare our experimental mixtures against. It is relatively undeveloped, annually disturbed by ice-rafts, is a non-serpentine upland environment in the stage of primary succession, and it is self-sustaining.

Generally, technosols used for site rehabilitation are dominated by a greater variety of quickly establishing grass species than is naturally found in reference sites

(Rowland *et al.,* 2009). However, we did not observe this in our study. In this study it was essential to include a variety of native nitrogen-fixing and non-fixing, forbs, herbs, grasses, and shrubs that were found along the Attawapiskat River floodplain in our seed mix because the company require the use of native plant species. Adding native plant species allowed us to generally examine the establishment of these early colonizing plant species on our experimental mixtures for mine rehabilitation. We did not test the viability on the seeds applied to our mixtures in 2014, however all treatments were treated the same. Out of the thirteen native plant species sown, six of them emerged. Although the average total plant cover, species richness, and species composition on our mixtures was significantly different from the natural environment of the Attawapiskat River floodplain, these results provide useful insight into the potential of our technosol mixtures to host native upland plant species during rehabilitation. There could be a variety of reasons why we did not see the emergence of some species, including potentially low viability, longer dormancy and germination periods of some species such as *Rosa acicularis* and *Physocarpus opulifolius*, unsuitable soil conditions, or wildlife predation.

Our experimental mixtures did share similar physical and chemical attributes with the natural environment, including soil bulk density, root penetration resistance, surface roughness, salinity (non-saline according to the USDA), and aboveground biomass. However, in some regards, chemistry was different between our technosols and the natural environment. Some of these differences can be attributed to the natural conditions of the mine waste material incorporated into our experimental mixtures, including alkalinity (Rowland *et al.*, 2009) and Ca:Mg ratio. Similar to peat-mineral mixtures evaluated in a study by Rowland *et al.* (2009), our technosols differed from the natural Attawapiskat River floodplain soil partially due to their nutrient status. Compared to the river soil, our experimental mixtures were more deficient in essential nutrients such as P, and were potentially more deficient in N. Since we did not test different rates of fertilizer application in our experiment, it may be difficult to determine if the deficiencies in P, and potentially N, were a major contributor to the vegetative differences between our experimental mixtures and the natural environment (Walker, 1954). The river soil also contained greater elevated concentration of heavy metals than our experimental mixtures. These results were surprising, because we expected there to be more elevated bioavailable heavy metals in our serpentine mixtures. Also, the C:N ratios of the natural river soil environment were more optimal than the C:N ratios of the raw materials used to construct our experimental mixtures.

The behavior of soil water content, temperature, and electrical conductivity in a soil profile can be indicative of soil aspects such as porosity, vegetation cover, organic matter content or chemistry; therefore, it is also important to consider these factors in comparison to natural conditions. We found the behavior of water movement and electrical conductivity in our mixtures was similar to that of the natural environment. In terms of maximum and minimum temperature changes, our experimental mixtures covered a more variable range throughout the year. Compared to the natural environment, our mixtures reached greater maximum and lower minimum temperatures, which may have limited plant growth. Soil surface temperatures greatly above and below 20 to 25 ^{o}C are capable of limiting or permanently damaging root growth, dependent on the tolerance of the plant species (Kramer, 1969). Soils, such as our experimental mixtures, where the surface is relatively less vegetated and more exposed, can reach much greater temperature extremes than more vegetated natural environments, such as the Attawapiskat River floodplain (Kramer, 1969). During the winter months, below ground temperature measurements indicated the formation of an insulating snow pack along the river floodplain. Snow cover accompanied by an abundance of low-lying herbaceous and shrub vegetation can create an insulating layer between the external environment and soil surface, conserving heat and protecting germinating seedlings from harsh conditions (Oke, 1987). Meanwhile, below ground temperatures in the South OB stockpile mix and our experimental mixtures were more variable (ranging from -5 to -17 ^{o}C). This suggested that less plant cover, a potentially thinner snowpack, and more exposure could have caused the rehabilitation mixtures to experience more extreme temperature changes than the natural environment. They could have been more susceptible to needle-ice formation during spring and autumn, as well as damage from more exposure during the winter months (Kramer, 1969; Oke, 1987).

We found that greater temperatures at the soil surface of our experimental mixtures resulted in slightly more accumulated growing degree-days than the natural environment. Growing degree-days are important indicators for plant establishment because they represent plant-temperature interactions that occur where above and below certain thresholds specific plants will not grow (Lowry, 1969). Surface exposure and the ultramafic physical properties of processed kimberlite within our mixtures could have increased surface temperatures resulting in more available growing days for establishing vegetation. In the environment, and specifically subarctic climates, this increase could be viewed positively as a factor to improve plant growth. However, the above-ground and below-ground accumulated growing degree-days of our mixtures did not vary greatly from our references sites during the first and second growing season. Each measurement was within, or slightly above the normal growing degree-days (750 to 1000) experienced in the vicinity of the De Beers Victor Mine (Agriculture and Agri-food Canada, 2014). However, growing degree-days in the vicinity of the De Beers Victor Mine are much lower than those encountered further south as they decrease further north. In southern Ontario, accumulated growing degree-days can range from 1600 to greater than 1800 (Agriculture and Agri-food Canada, 2014). Less accumulated growing degree-days can be considered another challenge associated with rehabilitation in subarctic environments.

Though our experimental mixtures were different in some regards to the Attawapiskat River floodplain, they did share similarities in several attributes. Understanding natural disrupted environments undergoing primary succession can be relevant for rehabilitation by examining interactions, and how they progress (Walker & Del Moral, 2009). Although our research only provides early results, our early results could indicate the potential of our experimental mixtures to eventually resembling an early successional environment similar to the Attawapiskat River floodplain. Eventually succession could progress to a well-developed upland environment with time.

Our understanding of technosols containing diamond mine waste would be improved from longer-term studies conducted on the influence of ultramafic processed kimberlite mine waste on vegetation establishment across our mixture gradient. With regards to other future studies, it may be beneficial to examine how establishing vegetation is influenced physiologically by the serpentine chemistry of our mixtures, such as examining uptake of calcium, magnesium, metals, or development of any adaptations. Shewry and Peterson (1975) explored this topic, and found plants colonizing on serpentine soils demonstrated selective transportation and accumulation of calcium and magnesium in their roots and shoots as an adaptation. It may also be beneficial to examine various applications of fertilizer to further examine the response of vegetation to nutrient deficiencies across the serpentine gradient we created.

Conclusion

Our findings suggest distinctions can be made between technosols containing non-serpentine and serpentine characteristics. This research suggested that our non-serpentine mixtures could have provided slightly better conditions for vegetation establishment compared to our serpentine mixtures containing processed kimberlite. However, most differences found between our mixtures were only minor. Factors that commonly limit plant growth, such available moisture and bioavailable calcium, could have been the main influence on vegetation establishment between our non-serpentine and serpentine mixtures. Though beneficial, in this experiment the quantity of peat did not influence vegetation establishment between our experimental mixtures. This could be explained by only a minor difference in organic matter between our 20% and 40% peat applications, causing them to behave similarly in our mineral mixtures.

Addressing stressors imposed by mine waste, through peat amendments and a native plant seed mix, was effective at improving conditions for vegetation establishment. This was evident when our mixtures were compared to un-amended mine waste controls. In addition, our mixtures did share several similar physical, chemical, and biological aspects with a peat-mineral mixture currently being used for rehabilitation at the Victor Mine, as well as the Attawapiskat River floodplain undergoing natural primary succession. Early similarities with these reference sites could show the potential of our mixtures to resembling an early successional environment similar to the Attawapiskat River floodplain, providing appropriate conditions for the establishment of native plant communities, capable of being used successfully for mine rehabilitation.

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Tables and Figures

Table 1. Native plant species used during seeding of each experimental mixture in 2014, including N-fixing forbs, N-fixing shrubs, forbs, grasses, and shrubs. Seeds were collected in 2014, but representative seed viability (%) is shown from seeds collected in 2016 (Rantala-Sykes & Campbell, unpublished). Population sizes for the seed viability tests are shown.

Functional Type	Species	Seed Viability (%)	Population Size (N)
Nitrogen Fixing Forbs	*Vicia americana*	98.2	6
Nitrogen Fixing Shrubs	*Alnus viridis*	55.7	4
Forbs	*Chamerion angustifolium*	64. 5	3
	Primula laurentiana	100	1
	Aquilegia brevistyla	95	2
	Achillea millefolium	95.1	3
Grasses	*Beckmannia syzigachne*	N/A	N/A
	Poa palustris	78.7	3
	Agrostis scabra	85.1	4
	Hierochloe odorata	64.2	4
Shrubs	*Physocarpus opulifolius*	53.6	4
	Rubus idaeus	92.1	6
	Rosa acicularis	77.14	3

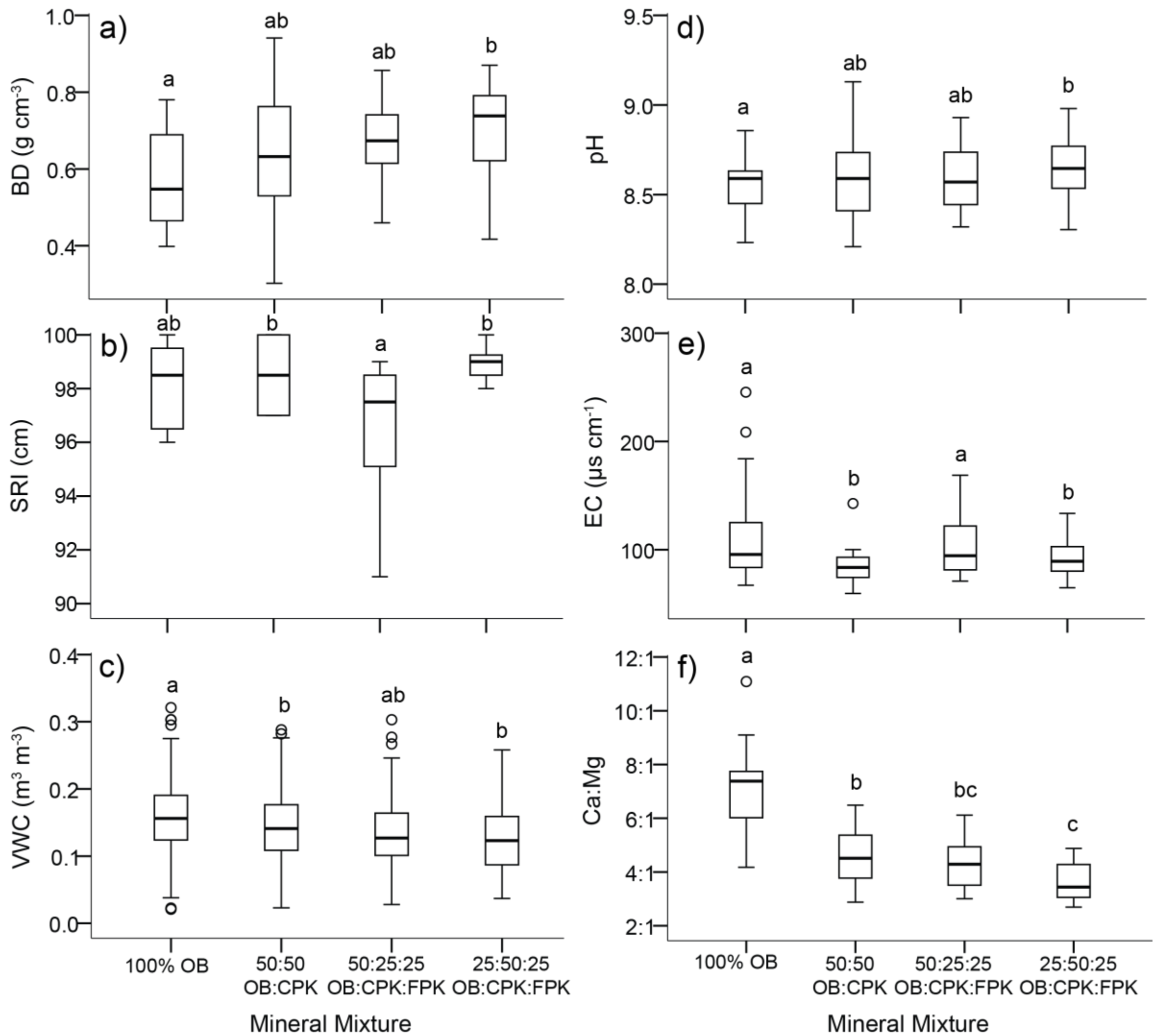

Figure 1. Box plots across the mineral mixture experimental treatments for a) bulk density (BD), b) surface roughness index (SRI), c) volumetric water content (VWC) from June to August 2015, d) surface pH, e) surface electrical conductivity (EC), and f) Ca:Mg ratio. Results of the post-hoc Tukey tests are shown.

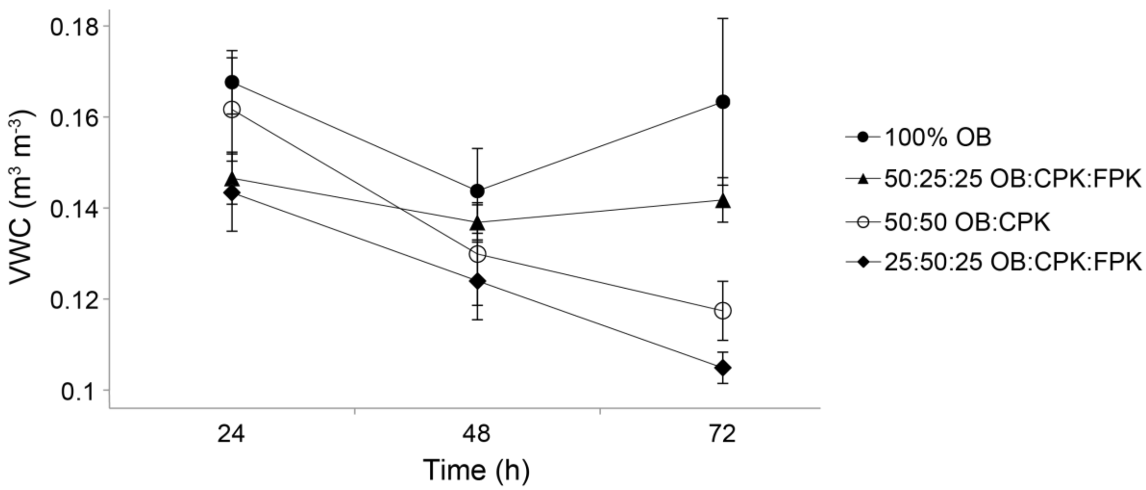

Figure 2. Mean volumetric water contents (VWC) for the mineral mixture experimental treatments over time of measurement. Measurements were taken 24, 48, 72 hours after a rainfall event ($P = 0.027$; $n = 144$).

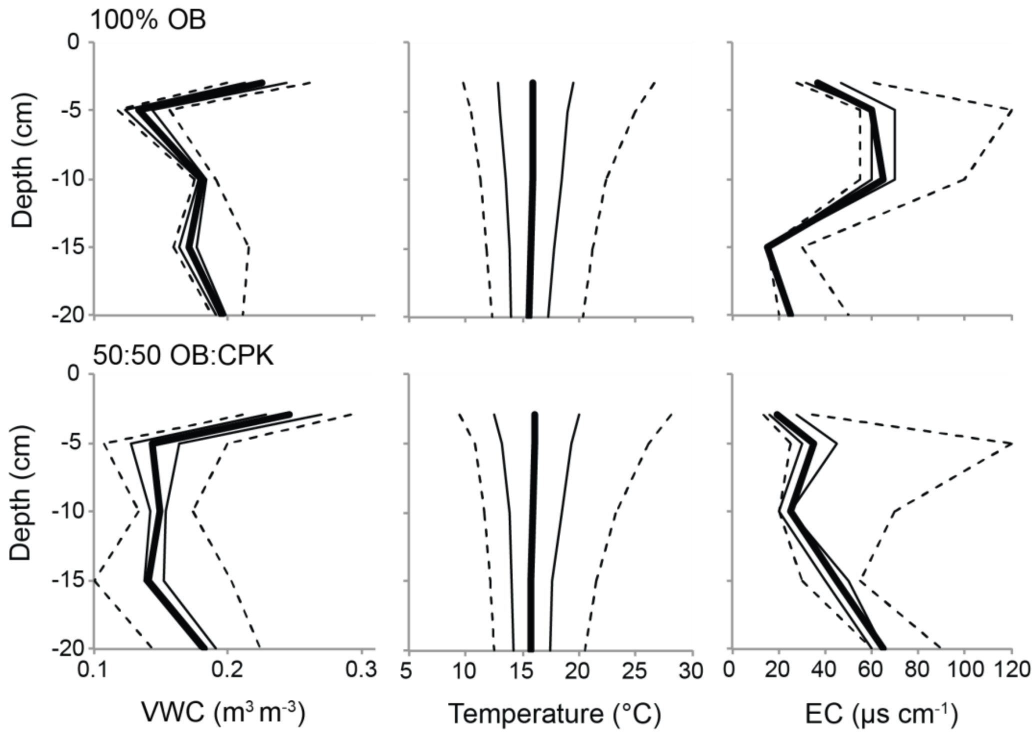

Figure 3. Variation in volumetric water content (VWC), temperature, and electrical conductivity (EC) of the 100% OB-20% peat and 50:50 OB:CPK-20% peat mineral mixtures with depth. Measurements were taken from July 21 to September 13, 2015. Dashed lines represent the 5% and 95% percentiles, thin solid lines represent the 25% and 75% percentiles, and thick solid lines represents the 50% percentile.

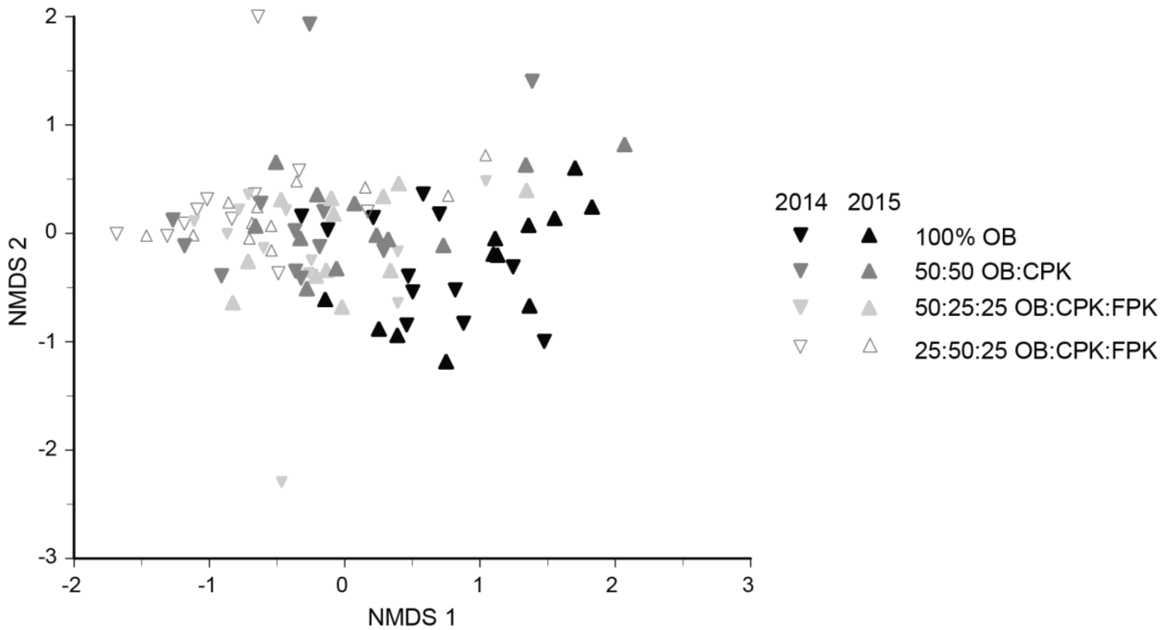

Figure 4. Non-metric multidimensional scale (NMDS) figure depicting the separation in bioavailable elements between the mineral mixture experimental treatments in 2014 and 2015 (Stress = 0.17).

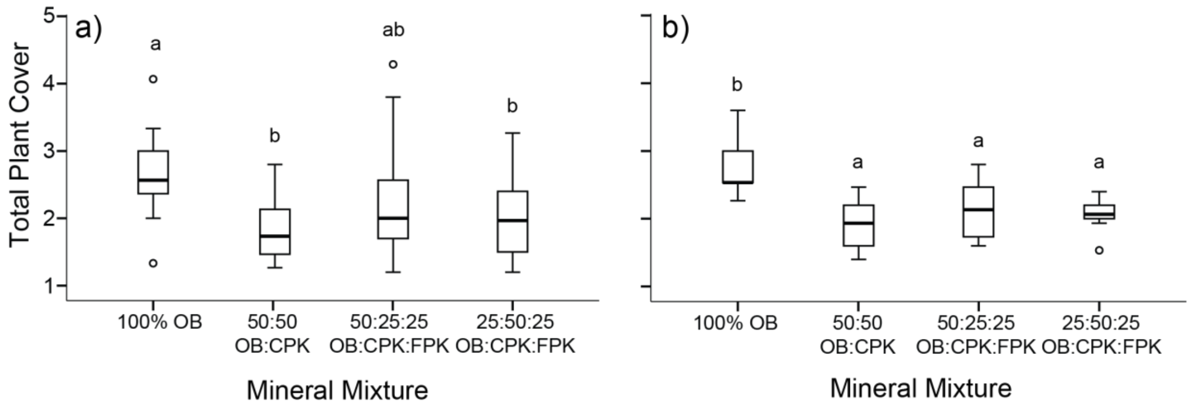

Figure 5. Total plant cover scores for the tops of the mineral mixture experimental treatments during a) August 2015, and b) August 2016 (1: < 1%, 2: 2-5%, 3: 6-20%, 4: 21-50%, 5: > 50%). Results of the post-hoc Tukey tests are shown.

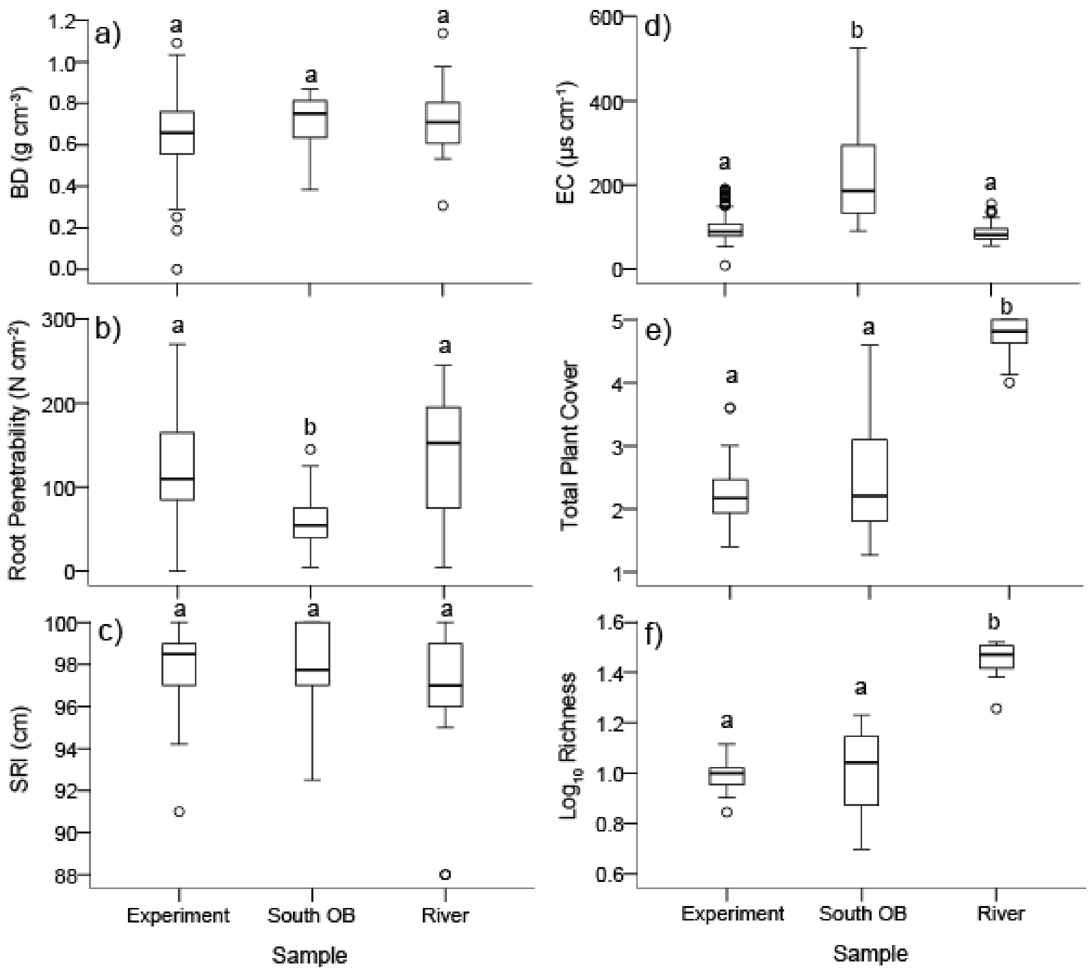

Figure 6. Boxplots comparing the experimental mixtures with the South OB stockpile and the Attawapiskat River floodplain reference sites, for a) bulk density (BD), b) root penetrability resistance, c) surface roughness index (SRI), d) electrical conductivity (EC), e) 2016 total plant cover score (1: < 1%, 2: 2-5%, 3: 6-20%, 4: 21-50%, 5: > 50%), and f) 2016 plant species richness. Results of the post-hoc Tukey tests are shown.

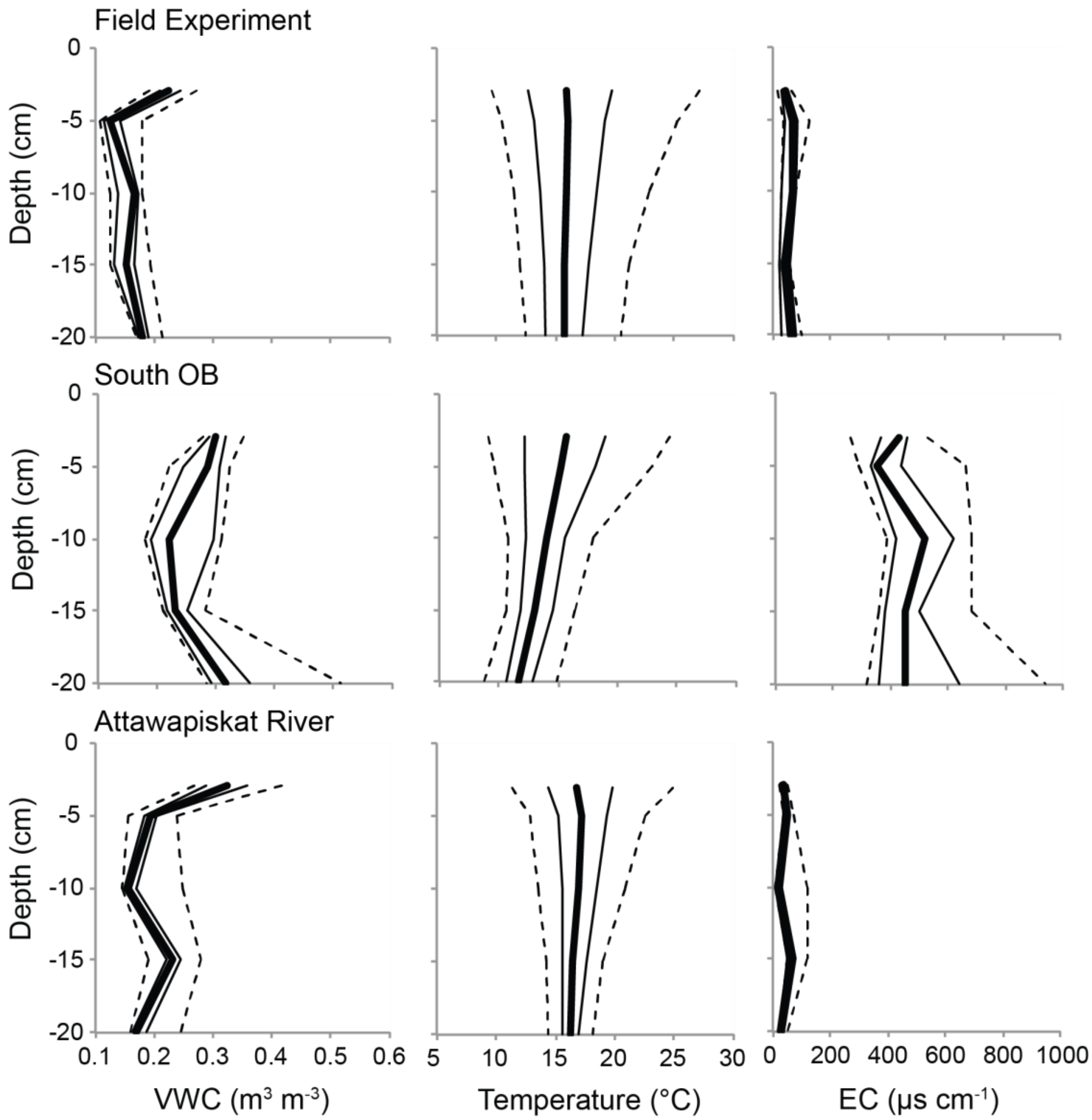

Figure 7. Variation in volumetric water content (VWC), temperature, and electrical conductivity (EC) of the field experiment mixtures, South OB stockpile, and Attawapiskat River soil with depth, from July 21 to September 13, 2015. Dashed lines represent the 5% and 95% percentiles, thin solid lines represent the 25% and 75% percentiles, and thick solid lines represents the 50% percentile.

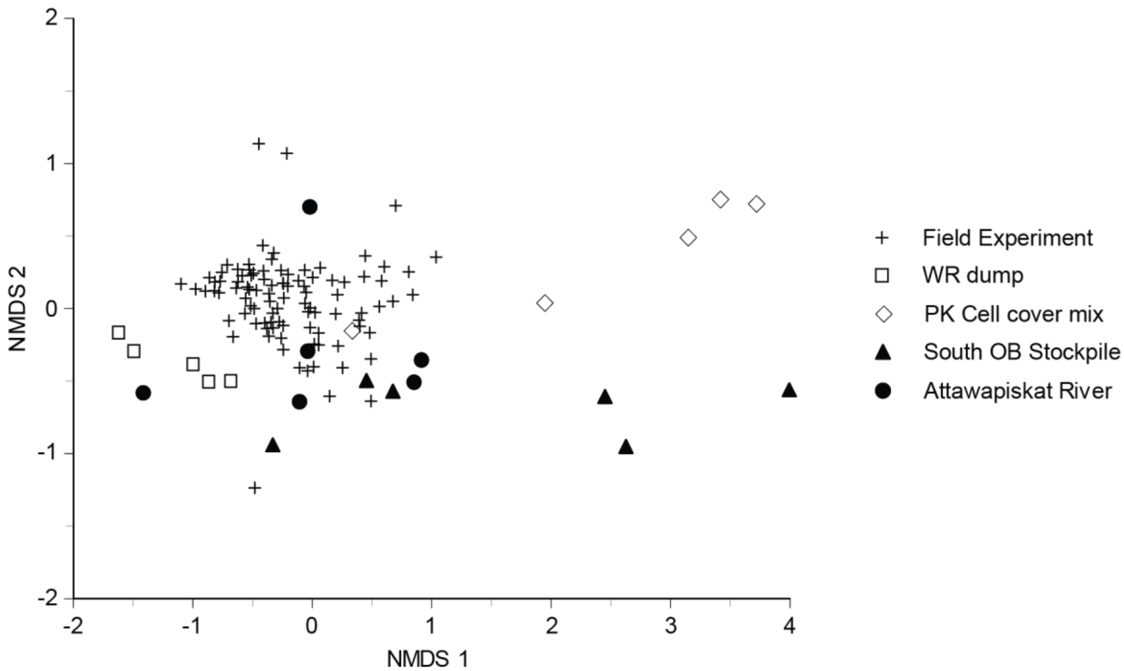

Figure 8. Non-metric multidimensional scale (NMDS) figure depicting separation in bioavailable elements between the field experimental mixtures, WR dump, PK Cell Cover mix, South OB stockpile, and Attawapiskat River floodplain reference sites in 2015 (Stress = 0.17).

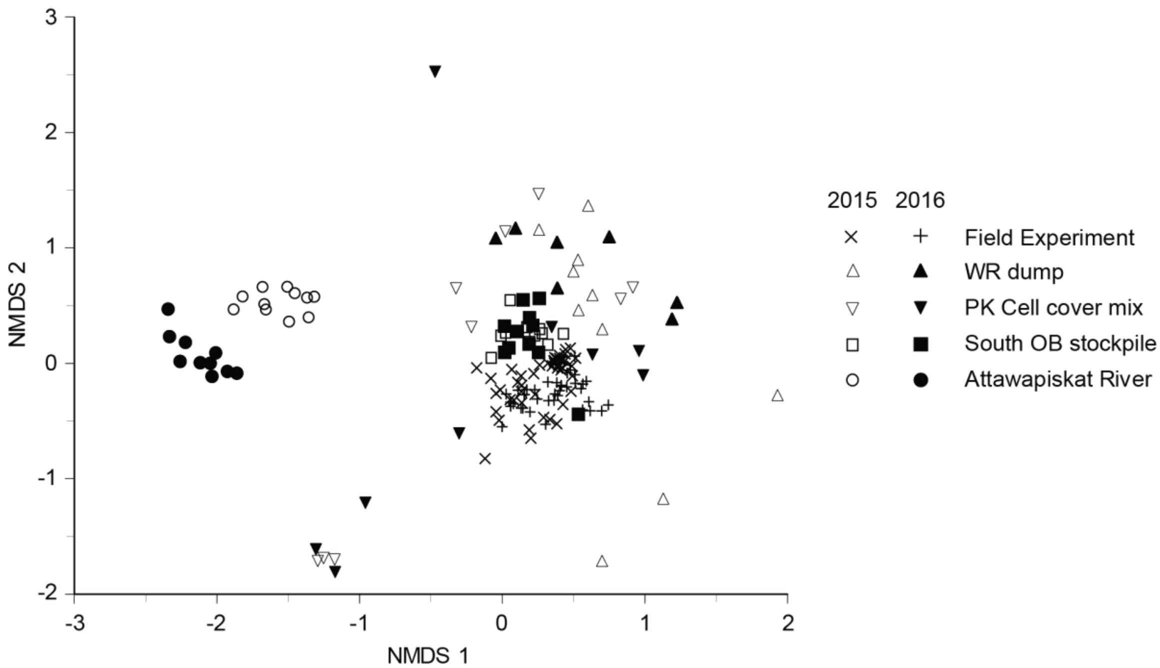

Figure 9. Non-metric multidimensional scale (NMDS) figure depicting separation in plant species assemblages between the field experimental mixtures, WR dump, PK Cell Cover mix, South OB stockpile, and Attawapiskat River floodplain reference sites in 2015 and 2016 (Stress = 0.17).

CHAPTER 3: Microbial activity of technosols constructed along a gradient with ultramafic diamond mine wastes

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Abstract

Soil microorganisms benefit plant productivity because of their role in biogeochemical cycling. This is especially important for nutrient-poor environments undergoing primary succession. Mine rehabilitation using technosols emulate these successional processes through improving the physical, chemical, and biological properties of barren substrates, including re-introducing microbial communities. The goal of this study was to examine the limitations on microbial colonization in technosols incorporating diamond mine wastes during rehabilitation, at the De Beers Victor Diamond Mine. Our objectives were to (1) determine whether mineral substrates of increasingly serpentine characteristics supported similar microbial activity, (2) determine whether increased peat amendments affected their microbial activity, (3) determine the extent to which the addition of simple sugars, as a proxy for available soluble carbon in the soil, stimulated microbial activity, and (4) examine how the microbial activity in this experiment compares to that in natural early succession substrates nearby. We constructed a 4 x 2 factorial field experiment on two areas of mine waste at the Victor Mine, a waste rock dumpsite, and a processed kimberlite storage facility. We constructed three test blocks on each site containing a total of 48 5 m x 5 m experimental plots with four mineral substrate mixtures, and two levels of peat application. Mixtures consisted of various combinations of coarse and fine Mg-rich processed kimberlite, a silty loam marine Ca-rich overburden, and 20% or 40% peat, creating an ultramafic, serpentine gradient. Each plot was mildly fertilized at a rate of 12.5 g m^{-2} (NPK 8-32-16), inoculated with a microbial inoculation ‘tea’ mixture containing local forest litter and N-fixing nodules, and seeded with a variety of native vegetation. We constructed an aerobic soil incubation experiment in growth chambers to examine the CO_2 respiration rates. Subsamples of these mixtures were examined three

times over a 7-day period after being incubated for 6 h on each sampling day. Our results indicated that mixtures with both non-serpentine and serpentine characteristics shared similar microbial activities by the end of the 7-day incubation period, however, early in the experiment, mixtures increasing in serpentine characteristics had statistically lower CO_2 respiration rates than non-serpentine mixtures. Minimal Differences in microbial activity by the end of the experiment could be due to only minor differences in physical and chemical properties (Chapter 2). The quantity of peat, and the addition of glucose, both had positive influences on microbial activity. In comparison to early successional reference environments, microbial activity was lower in our mixtures. Our study only provides us with early results, however, the early similarities we found in our field study (Chapter 2) seem to show the potential of our experimental mixtures to resembling an early successional environment similar to the Attawapiskat River floodplain, able to host similar native upland plant species and eventually develop similar microbial activity. Further research should be conducted, on the long-term influence, differences in microbial communities, and physiological influences on colonizing communities to gain a better understanding of technosols containing diamond mine waste.

Key Words: serpentine soil, processed kimberlite, anthroposol, mine reclamation, microorganisms, primary succession, soil incubation

Abbreviations

HBL: Hudson Bay Lowland

OB: silty loam overburden

CPK: coarse processed kimberlite

FPK: fine processed kimberlite

Introduction

Soil microbial communities play a crucial role in bio-geochemical cycling of nutrients for terrestrial plant communities, and can be important regulators of plant productivity (van der Heijden *et al.*, 2008; Hahn & Quideau, 2013; Beasse *et al*., 2015). Soil microorganisms provide plants with essential nutrients through decomposition, nitrogen fixation and mycorrhizal scavenging of phosphorus, and in return receive carbon sources for respiration (van der Heijden *et al.*, 1998; Batten *et al.*, 2006; van der Heijden *et al.*, 2008). Microbes are also capable of mobilizing microelements essential for plant growth and immobilizing heavy metals in contaminated conditions (Gadd, 2004). Microbial biomass and activity are good indicators of overall soil quality in contaminated landscapes (Giller *et al.,* 1998; Wang *et al.*, 2007). However, microbial biomass and activity are sensitive to soil texture (Hamarshid *et al.*, 2010), shifts in temperature, soil moisture, pH, redox potential, organic matter, the content of essential elements (Fierer & Jackson, 2006; Ferreira *et al.*, 2013; Hahn & Quideau, 2013), and shifts in plant communities (Batten *et al.*, 2006; Beasse *et al.*, 2015).

Soil resources such as organic matter, essential nutrients, and biota are minimal in barren substrates, creating a challenging environment for colonization. Microbes and vegetation slowly colonize these substrates and build up these soil resources through processes of primary succession (Walker & Del Moral, 2009; Yoshitake *et al.*, 2016). Plant, microbial and mycorrhizal communities shift and transform together, influencing the development of these barren environments (Hobbs *et al.*, 2007). Soil microorganisms benefit plant productivity, especially in early successional, nutrient-poor environments, because of their role in biogeochemical cycling (van der Heijden *et al.*, 2008). Although primary succession is naturally a slow process (Forbes & Jefferies, 1999), rehabilitation managers can emulate and speed up this natural process by improving the physical, chemical, and biological properties of the barren substrates to be able to host biological life, through adding organic matter and a biological component (Bradshaw & Chadwick, 1980; Drozdowski *et al*., 2012; Brown & Naeth, 2014), including the re-introduction of microbial communities (Sheoran *et al.*, 2010). Mines, for instance, produce large areas of barren substrates that must be rehabilitated at mine closure. They may be rehabilitated by combining salvaged mineral and organic soils and suitable mine waste substrates (McMillan *et al.*, 2007). Available materials often lack of available nutrients, texture, a suitable structure, soil organic matter, and can have limiting chemical characteristics, such as the presence of heavy metals (Bradshaw & Chadwick, 1980; Wong, 2003; Drozdowski *et al.*, 2012; Naeth & Wilkinson, 2014). Suitable substrates may be especially difficult to obtain for mines in remote region.

Total carbon content largely influences microbial activity within soils (Pessoa-Filho *et al.*, 2015), so mineral mine wastes are often mixed with organic matter (such as peat) during soil rehabilitation, as was done during the rehabilitation of bitumen-mined landscapes in boreal Alberta (McMillan *et al.*, 2007). Mixing peat into a barren mineral substrate was found to not only improves soil structure, but also fosters water and nutrient retention and availability (Danielson *et al.*, 1983a; Larney & Angers, 2012). The addition of peat was also found to improve microbial activity within the soil by adding an external source of microbes to the new environment (Danielson *et al.*, 1983b; Fernández *et al.*, 1999). This accelerates the cycling and retention of nutrients essential for plant growth, such as carbon, nitrogen, and phosphorous (Danielson *et al.*, 1983b; Fernández *et* *al.*, 1999; DeGrood *et al*., 2005; van der Heijden *et al.*, 2008; Hahn & Quideau, 2013). Applying forest floor litter to mineral substrates can have an even more beneficial outcome on microbial and plant communities than applying peat alone (McMillan *et al.*, 2007; Larney & Angers, 2012). Forest floor litter accelerates the establishment of microbial and plant communities of local natural forests (Hahn & Quideau, 2013).

The mineral component of rehabilitated substrates can also dictate microbial activity. The ultramafic kimberlite tailings associated with diamond mining (Webb *et al.*, 2004) can provide challenging conditions for plants and microbes, hosting similar physical and chemical characteristics to ultramafic or serpentine soils (Reid & Naeth, 2005; Hobbs *et al.*, 2007; Drozdowski *et al.*, 2012). Serpentine soils form from weathering of ultramafic parent material, and often host unique plants and plant associations, exhibiting tolerances and adaptations to their challenging conditions (Fitzsimons & Miller, 2010; Doubková *et al.*, 2012). They often lack clay and silt, so as a result are more susceptible to drought conditions and weak nutrient retention (Brady *et al.*, 2005). They also contain elevated concentrations of metals (i.e. Fe, Ni, Cr, Co), have low calcium to magnesium ratios and are deficient in essential nutrients (Brady *et al.*, 2005). Serpentine soils not only influence plant establishment, but may also influence microbial communities. Mycorrhizae species differ between serpentine and non-serpentine soils (DeGrood *et al.*, 2005; Rajakaruna *et al.*, 2009). Elevated heavy metal concentrations in serpentine soils can have a negative influence on microbial diversity, biomass, and activity (Fernández *et al.*, 1999; Schipper & Lee, 2004); although, microbial communities may develop tolerances if the bioavailability of metals is high (Schipper & Lee, 2004; DeGrood *et al.*, 2005).

We conducted an incubation experiment to better understand the limitations for microbial colonization of subarctic diamond mine wastes during mine site rehabilitation. We had previously created an experimental field gradient of mineral substrates from Ca-rich overburden deposits to Mg-rich processed kimberlite, with factorial combinations of peat. These mixtures were fertilized mildly and inoculated with a 'tea' made from forest litter and N-fixing nodules. In the current factorial incubation study, we wanted to test (1) whether mineral substrates increasing in serpentine characteristics supported similar microbial activity, (2) whether increased peat amendments affected their microbial activity, (3) the extent to which the addition of simple sugars, as a proxy for available soluble carbon in the soil, stimulated microbial activity, and (4) how the microbial activity in this experiment compared to that in natural early succession substrates nearby. We hypothesised that the presence of serpentine characteristics has a negative influence on soil microbial activity and that peat amendments improve microbial activity. In addition, we hypothesised that the addition of a simple sugar to peat-mineral mixtures does improves microbial activity.

Materials and Methods

STUDY AREA

The De Beers Victor Diamond Mine is located within the Hudson Bay Lowland (HBL; 52° 49’08” N 83° 54’52” W). The HBL is the third largest wetland in the world, spanning an area of 373 700 km^2 (Abraham & Keddy, 2005). It is a Paleozoic limestone plain, overlain, in the area of the mine, by recent marine sediments, from < 5 m to 30 m in depth and capped by an ~ 2 m thick of peat (Martini *et al.*, 2006; Riley, 2011), resulting in a landscape that is very flat and poorly drained (Abraham & Keddy, 2005). It has a humid, high boreal eco-climate that is greatly influenced by its proximity to Hudson Bay and James Bay and is characterized by short cool summers and long cold winters. Permafrost is discontinuous. Peatlands, specifically bogs and fens, dominate the terrain, and isolated uplands account for ~ 2% of the landscape (Riley, 2003; Abraham & Keddy, 2005).

The De Beers Victor Diamond Mine is an open pit mine that began operation in 2008, with a production lifespan estimated at ~ 11 years. Upon closure, the Victor Mine and its wastes will be rehabilitated toward a well-drained upland environment with low-forested hills (De Beers Group of Companies & AMEC, 2014).

EXPERIMENTAL DESIGN AND SAMPLING

We constructed a factorial experiment during the summers of 2013 and 2014 (see Hanson & Campbell, in prep. for details). We created three experimental blocks on each of two areas of mine waste at the Victor Mine, a limestone waste rock dumpsite (WR dump), and a processed kimberlite storage facility (PK Cell). Each block contained eight 5 m x 5 m plots (n = 48). We used three available mineral mine waste substrates to make mineral mixes; namely, marine overburden (OB), coarse processed kimberlite (CPK) and fine processed kimberlite (FPK; Table 1). We created four mineral mixes: (i) 100% OB; (ii) 50:50 OB:CPK; (iii) 50:25:25 OB:CPK:FPK; and (iv) 25:50:25 OB:CPK:FPK. This created a gradient of 100% OB to a mineral mix of 75% kimberlite. To these, we mixed in either 20% or 40% of peat, for a total of 4 x 2 factorial treatments. In late summer 2014, we created a ‘litter tea’ for microbial inoculation for each plot. We collecting ~ 4.5 L of each upland forest litter and humus from under spruce trees (*Picea glauca* or *P.*

mariana), under buffaloberrry (*Shepherdia canadensis*) and under alder (*Alnus crispa* var. *viridis* or *A. incana* var. *rugosa*); added 1 to 4 crushed rhizobium nodules from *Vicia americana* and *Alnus* sp.; steeped it in 54 L of room temperature water for ~ 12 hours; then strained the mixture through a 2 mm mesh sieve. We applied ~ 6 L of this inoculant 'tea' to each plot. We fertilized the soil mixtures at a rate of 12.5 g m^{-2} with a dry blend fertilizer (8-32-16 NPK) and then we seeded each mixture with a variety of native nitrogen-fixing shrubs and herbs, and grasses, forbs, and shrub seeds collected locally during 2014 (Chapter 2).

In late August 2015, we took five soil surface subsamples of the top 10 cm at five locations within each experimental plot (n = 8 treatments x 6 replicates = 48) using a 2.5 cm diameter soil core and bulked the five subsamples. As controls, we also sampled raw OB (n = 3) and raw peat (n = 3). We also sampled two early successional reference sites, including a reclaimed overburden stockpile covered with a 30 to 50 cm thick 1:1 cover mixture of peat and silty loam OB, that was created in 2011 and left un-seeded (South OB stockpile; n = 3), and substrates along the Attawapiskat River floodplain with early plant colonization (n = 3).

We conducted an aerobic soil incubation experiment in November 2015 to measure microbial activity of each sample following the methods of Noyce *et al.* (2015), with several modifications. We incubated two sets of each sample, with 40 g of material in 250 mL mason jars. We added glucose, at a rate of 1 mL of a 20 mg mL^{-1} glucose solution to one set of the samples. Prior to the incubations we dried a portion of each soil sample at 105 ^{o}C for 24 hours in a Fisher Scientific® Isotemp drying oven, then weighed them on a Denver® digital balance (Model: P1-2002) to determine the maximum soil water content of all of the samples. We then calculated and applied the amount of water required to bring each sample to approximately the same soil water content using the maximum water content we found. We incubated them in a growth chamber, in the dark with the temperature set to 15 ^{o}C to mimic the average temperatures observed at the De Beers Victor Diamond Mine from May to September 2015. We examined respiration rates over a weeklong experiment, on day 0, 3, and 7, after 6 hours of incubation. We processed gas samples using a SRI model 8610C Gas Chromatograph® analyzer and PeakSimple® software. Several incubations gave slight negative respiration results, but these were kept in the analyses because they were close to the sensitivity of the gas chromatograph.

We analyzed raw mineral mine waste materials and peat for total nitrogen and sulphur content using an Elementar Vario EL Cube CNS analyzer at LUCAS Services in Thunder Bay, ON. Due to the high concentration of calcium from $CaCO_3$ in the raw materials, total organic carbon content was also determined using LOI values using a conversion (Total C = (0.443 x LOI) - 2.77) suggested by Wright *et al.* (2008). Total elements for the raw materials were determined from aqua regia dissolution followed by ICP-MS (Bergeron & Campbell, unpublished). Bioavailable nutrient analyses on the raw materials and mineral mixtures were prepared using $LiNO_3$ extractions at the Elliot Lake Research Field Station and were determined with ICP-MS, following the methods of Abedin *et al*. (2012) (Bergeron & Campbell, unpublished).

STATISTICAL ANALYSIS

We analyzed the respiration data from the incubation experiment using a factorial design analysis of variance using SPSS®, version 21, with waste sites and blocks as random factors, and mineral mixtures, peat and glucose addition as fixed factors. We analyzed each time period separately. In a separate analysis of variance, we compared the respiration rates of the 100% OB-peat mix from the field experiment with our controls of raw OB and raw peat. In a final analysis of variance, we compared the entire field experiment to the two early successional reference sites. We graphed residuals to verify assumptions of the analyses. We used post-hoc Tukey tests to examine the significant results. The type I error rate was set at 5%, and considered those within +10%.

Results

All the raw mineral materials in our experiment were alkaline (Table 1). There is also a clear calcium to magnesium ratio (Ca:Mg) gradient across our serpentine mixtures, that increased in their amount of processed kimberlite. Our 100% OB-peat mixes had the highest Ca:Mg ratios (mean 7.2:1), followed by our 50:50 OB:CPK-peat and 50:25:25 OB:CPK:FPK-peat mixes (mean 4.6:1 and 4.3:1, respectively), whereas our 25:50:25 OB:CPK:FPK-peat mixtures had the lowest Ca:Mg ratio (mean 3.6:1). The processed kimberlites also had elevated total Fe, Ni, Mn, Cr, Cu, Co, and Se (Table 1). Total Ni, Cr, and Se of the OB, CPK and FPK exceeded totals from the Canadian Council of Ministers of the Environment (CCME) industrial soil quality guidelines. The carbon to nitrogen (C:N) ratio of the raw peat was the lowest (mean 22:1), followed by OB (123:1), FPK (194:1), and CPK with the greatest C:N ratio (mean 313:1). In comparison to our experimental mixtures, the reference sites were also quite alkaline and had higher Ca:Mg ratios (13:1 and 12:1 respectively; Table 2). The C:N ratios of the river and South OB site were 5:1 and 22:1, respectively (Table 2).

In this experiment, each of the mineral mixtures had significantly different respiration rates on day 0 and day 3, but not on day 7 (P = 0.013, P < 0.001, and P = 0.535, respectively; Table 3; Figure 1a). Those containing 100% OB had higher respiration rates than the mixtures containing 50:25:25 OB:CPK:FPK and 25:50:25 OB:CPK:FPK on day 0 and 3.

The mixtures containing 40% peat also had higher respiration rates than the mixtures containing 20% peat on day 0 and day 3 (P = 0.006, and P = 0.006, respectively; Table 3; Figure 1b), with borderline significance on day 7 (P = 0.065; Table 3; Figure 1b). The addition of glucose produced higher respiration rates on days 3 and 7 (P < 0.001 and P < 0.001, respectively; Table 3) than the samples lacking glucose, but there were no interactions amongst mineral mixtures, peat content and glucose additions.

When we compared our OB-peat mixtures with raw OB and raw peat, we found a significant difference in respiration rate on day 0 ($F_{2, 29}$ = 13.239; P < 0.001), with raw OB having the lowest respiration (mean -0.426 μg CO_2 g dry soil^{-1} day^{-2}), raw peat having the highest respiration rate (mean 9.145 μg CO_2 g dry soil^{-1} day^{-2}) and the OB-peat mixtures from the field experiment having intermediate values. This difference disappeared by day 3 and 7 ($F_{2, 30}$ = 0.525 and P = 0.597; $F_{2, 30}$ = 1.375 and P = 0.268). Glucose increased respiration on days 0, 3 and 7 ($F_{1, 29}$ = 4.199 and P = 0.05; $F_{1, 30}$ = 8.512 and P = 0.007; $F_{1, 30}$ = 7.490 and P = 0.01), and an interaction was present between the material and glucose addition on day 0 ($F_{2, 29}$ = 6.048; P = 0.006), whereas raw peat experienced the greatest response in respiration rate with the addition of glucose while raw OB and our OB-peat mixture experienced little change with the addition of glucose.

When respiration from the field experiment was compared with the early successional reference sites, the river soil experienced the highest respiration rates on day 0 and day 3, whereas on day 7 the South OB stockpile experienced greater respiration rates (each $P < 0.001$; Table 4; Figure 1c). All sites had lower respiration rates with the absence of glucose, but there were interactions between glucose addition and these sites on day 0 ($P < 0.001$; Table 4), day 3 ($P = 0.028$; Table 4), and day 7 ($P < 0.001$; Table 4). On day 0, the river had four times the respiration rate with glucose addition, shifting from a mean of 15.6 to 70.0 $\mu g\ CO_2\ g$ dry soil^{-1} day^{-2}, whereas on day 3 the river soil experienced a slight decrease in respiration rate with glucose, from a mean of 41.4 to 30.7 $\mu g\ CO_2\ g$ dry soil^{-1} day^{-2}. On day 7 the South OB stockpile shifted from a low respiration rate 4.4 to 158.4$\mu g\ CO_2\ g$ dry soil^{-1} day^{-2} with the addition of glucose.

Discussion

The incubation experiment did not fully support our first hypothesis. By the end of the 7-day period, we found no difference in microbial CO_2 respiration rate between our non-serpentine and serpentine mixtures. This occurred despite research suggesting microbial activity is inhibited by elevated heavy metals (Giller *et al*., 1998), and despite the moderately high bioavailability of some elements (Ni, Cu, Mn) in our experimental mixtures (Chapter 2). Our results suggest that increasing quantities of processed kimberlite in the mixtures may not have inhibited microbial activity in the long-term, over the week-long incubation, but may have had a brief short-term response. The short-term microbial response, and lack of long-term response may be related to specific chemical properties of our mineral mixtures.

There is currently little research relating microbial activity to processed kimberlite; however, microbial response to serpentine conditions has been researched (Fernández *et al.*, 1999; Schipper & Lee, 2004; De Grood *et al.*, 2005; Pessoa-Filho *et al.*, 2015). Microbial communities are important components of serpentine soils due to their ability to access essential nutrients (Smith & Read, 2008, as cited by Fitzsimons & Miller, 2010). Chemically, serpentine soils can be challenging for vegetation establishment (Whittaker, 1954; Brady *et al.*, 2005). They are characterised for their infertility and also for their higher concentrations of magnesium (Whittaker, 1954; Brady *et al.*, 2005), which was evident in this experiment. All of our experimental mixtures (serpentine and non-serpentine) had low concentrations of essential nutrients such as N, P and K (Chapter 2), which may have been a factor that negatively influenced microbial activity across all of our mixtures. These nutrients are also naturally deficient in subarctic regions (Johnson & Van Cleve, 1976) and in mineral mine waste substrates (Johnson & Van Cleve, 1976; Bradshaw, 1996; Wong, 2003; Hobbs *et al.*, 2007). Our field study (Chapter 2) found there was minimal difference in the elevated bioavailable metals (Ni, Cu, Mn) in our experimental mixtures, which may have also been a factor resulting in minimal difference in microbial activity between our mixtures over the week-long incubation. On the other hand, several studies have found that microbial activity in serpentine soils was similar to non-serpentine soils even with elevated concentrations of heavy metals present (Schipper & Lee, 2004; Pessoa-Filho *et al.*, 2015). It was determined that microbial communities are capable of becoming tolerant to stresses imposed by the heavy metals in ultramafic material (Schipper & Lee, 2004). It is suggested that microbial communities that are well adapted to heavy metals within serpentine soils may actually improve plant establishment in these conditions by helping plants adapt to heavy metals (Vivas *et al.*, 2003, as cited by Fitzsimons & Miller, 2010).

Research conducted specifically on microbial communities inhabiting serpentine soils found that overall abundance of microorganisms did not differ between serpentine and non-serpentine soils; however, the microbial species were different (Oline, 2006). We may have experienced this in our study, but were unable to determine this as we only assessed microbial respiration. Further research should be conducted to examine specifically the microbial communities present within our non-serpentine and serpentine mixtures. It would be interesting to distinguish potential differences in microbial function across the serpentine gradient and examine their influence on vegetation establishment.

The short-term higher respiration rates in the non-serpentine (100% OB) mixtures may be related to their physical properties in comparison to mixtures increasing along the serpentine gradient. Although soil water content was controlled for during the incubations, moisture is recognized as an important regulator of microbial activity. Studies have demonstrated that respiration increases as soil water content increases (Signh & Gupta, 1977; Illeris & Jonasson, 1999; Davidson *et al.*, 2000). Our non-serpentine (100% OB) mineral mixtures were dominated by silt sized particles and were able to retain more water than the mixtures harbouring the most serpentine characteristics (25:50:25 OB:CPK:FPK) that contained 50% medium sand (Chapter 2). Serpentine soils are also capable of depressing microbial activity in the initial stages of their development by their low water content and nutrient retention (Brady *et al.*, 2005; Alexander *et al.*, 2006; Sylvain & Wall, 2011). Over time these soils weather further and are able to support more plant communities, thus creating a better environment to support microorganisms (Alexander *et al.,* 2006). Our incubation study further supports the findings from our field study Hanson and Campbell (Chapter 2), suggesting that our non-serpentine mixtures may not only possess beneficial properties to support plant establishment, but also better support microbial communities than mixtures with increasing serpentine characteristics. However, differences in the performance of our experimental mixtures were only minor, similarly to that determined by Hanson and Campbell (Chapter 2).

This incubation experiment supported our second hypothesis, that the quantity of peat applied to our mixtures had a positive influence on microbial activity. Both non-serpentine and serpentine mineral mixtures containing 40% peat exhibited higher respiration rates than those containing 20% peat, on all sampling days. These results support several other studies that found microbial activity increased when more organic material was available for decomposition (Fernández *et al.*, 1999; Schipper & Lee, 2004; DeGrood *et al.*, 2005; Pessoa-Filho *et al.*, 2015). Early successional environments, such as our experimental mixtures, require organic amendments to introduce nutrients in order to stimulate microorganisms activity (DeGrood *et al.*, 2005; Yoshitake *et al.*, 2016). Also, organic matter may have improved water-holding capacity to aid microbial communities (De Grood *et al.*, 2005; Larney & Angers, 2012).

However, Hanson and Campbell (Chapter 2) determined there was no difference in organic matter between our 20% and 40% peat applications, contrary to what we attempted to achieve in this experiment (Chapter 2). The minimal difference between our attempted 20% and 40% peat applications may have potentially resulted in only minor respiration rate differences between the peat applications. Therefore, we are unable to confidently determine from this study that these differences in microbial activity were due to the peat applications we attempted to achieve. During the construction phase of the project we had to consider the depth that the disk harrow was able to mix when we applied the peat amendments. Considering this depth, the difference between our 20% and 40% peat applications was only ~ 4 cm. Once tilled into each mixture the difference in peat applications could have diminished further; tilling could have pushed peat towards the edges and down the sides of the experimental mixtures.

Although we did not test our mixtures without the addition of peat, our study does support the positive influence peat has on microbial activity. Our 100% OB-peat mixtures had intermediate respiration rate values when compared to mineral and organic mine waste controls, raw peat and raw OB, indicating that combining peat with mineral mixtures is beneficial to microbial activity. However, this difference was short-lived. Previous research found similar results. Using peat specifically as an organic amendment was found to improve microbial activity by adding a carbon source, and may have potentially added an external source of microbes to the new environment (Danielson *et al.*, 1983b; Fernández *et al.*, 1999; van der Heijden *et al.*, 2008; Hahn & Quideau, 2013). In future studies, applying even more peat in combination with the forest floor litter inoculant could potentially improve microbial activity within our experimental mixtures. As suggested by McMillan *et al.* (2007) and Larney and Angers (2012), peat in combination with forest floor litter had more of a positive influence than the application of peat alone. Therefore, our results could also suggest that adding forest floor litter, but in the form of a microbial inoculation mixture, was effective at improving microbial activity within our mixtures.

Glucose dramatically influenced microbial activity at all stages of our experiment, supporting our third hypothesis. Microbial activity within soil is also largely influenced by the availability of soluble carbon substrates, which are supplied by vegetation in the natural environment (Jonasson *et al*., 1996 as cited by Martínez-Trinidad *et al.*, 2010). In comparison to microbial demand, carbon substrates within soils are generally in short supply, but can be improved through the use of organic amendments (Ros *et al*., 2003), such as peat, as was used in our experiment. As an alternative, soluble carbon sources, such as glucose, can also be used to improve microbial activity (Martínez-Trinidad *et al.*, 2010; Ferreira *et al.*, 2013). Glucose is added to enhance microbial activity is short field studies to mimic the root exudates that would naturally be released in the rhizosphere. How microbial communities respond to the added soluble carbon provides insight into factors that can limit activity. The positive response of microbial activity in this experiment was similar to findings from other studies involving glucose addition (Illeris & Jonasson, 1999; Schmidt *et al.*, 2000; Martínez-Trinidad *et al.*, 2010). This suggests microorganisms were able to easily acquire the soluble carbon that was added to our mixtures because it was a limited resource. The lack of interaction between the microbial response in our non-serpentine and serpentine mixtures with the addition of glucose could suggest that our mixtures were equally deficient in available carbon substrates.

The glucose experiment enhanced and stimulated interactions between glucose addition and reference site material. When we compared our mixtures to the raw peat control, we noticed a dramatic increase in respiration rate from the raw peat in comparison to the other materials when glucose was added. Microbial communities could have initially responded positively to the already existing carbon energy storage in the raw peat, which could have further enhanced their response when glucose was added (Larney & Angers, 2012). A similar situation may have also occurred with soil samples from the Attawapiskat River floodplain. Shortly after the addition of glucose, at the beginning of the experiment, the river floodplain substrate experienced a dramatic microbial response. Heightened respiration rate shortly after the addition of glucose could indicate the natural carbon was limited in this environment, but most likely contained more microbial biomass. As the experiment progressed, microorganisms could have quickly exhausted the available carbon. Martínez-Trinidad *et al.* (2010) found similar results; a rapid exhaustion of carbon may have led to the decrease in respiration rate observed from the river soil during the remainder of our incubation experiment. Near the end of our experiment the respiration rate from the river soil approximated that of our experimental mixtures. Throughout the incubation experiment the respiration rate from our experimental mixtures slowly increased, indicating a low microbial biomass and slow growth of these microbial communities. A dramatic increase in respiration rate from the South OB stockpile after the addition of glucose was not experienced until near the end of the incubation experiment, indicating a lag in microbial consumption; indicating low microbial biomass or dormant microbes.

Microbial communities are recognized as an important component in the establishment of sustainable ecosystems (DeGrood *et al*., 2005). Therefore, they are important to consider when constructing technosols for mine site rehabilitation. Improving microbial activity in technosols is positively correlated to vegetation success

(DeGrood *et al.*, 2005; Hahn & Quideau, 2013). The success and performance of technosols should also be evaluated based on their resemblance to, and potential to become, similar to reference environments. The goal of rehabilitation at the De Beers Victor Mine is to create a well-drained environment that is able to host a variety of native upland vegetation. In this experiment, the South OB stockpile represents rehabilitation efforts currently underway at the Victor Mine, while the Attawapiskat River floodplain represents a natural upland environment undergoing primary succession. For these reasons, both are ideal reference sites with which to compare our mixtures. Studying components of natural primary succession ecosystems promotes a better understanding of how to construct a new environment on barren substrates (Bradshaw, 1996).

The mixtures in our study had similar microbial activity to the South OB stockpile mix during most of the incubation experiment, which could be related to similarities in total plant cover and species richness as observed in our field study (Chapter 2). Although it was un-inoculated and un-seeded compared to our experimental mixtures, the South OB stockpile mix had several additional years to establish a natural vegetation cover and naturally established microbial communities.

Compared to the Attawapiskat River floodplain soil, our mixtures tended to have lower microbial activity. The natural river floodplain had more established plant communities than our mixtures, as indicated by total plant cover and species richness (Chapter 2). Areas with more established plant cover there experience more organic matter input and potentially greater microbial community diversity (Hahn & Quideau, 2013). Our mixtures contained greater concentrations of magnesium in comparison to the river soil. As a result, this may have influenced microbial community function. The river soil did contain greater concentrations of essential nutrients N and P compared to our mixtures (Chapter 2). This could have benefitted microbial communities. The river soil also contained elevated bioavailable metals and some elements, Fe, Ni, Mn, Cr, and Co, than our mixtures, but did not appear to impede microbial activity (Chapter 2). Our findings were similar in some ways to another study, Hahn & Quideau (2013), which found microorganisms in technosols differed from natural soils, largely owing to soil-plant interactions. Microbial activity in rehabilitated environments was found to be generally low but tended to increase as plant communities develop (Hahn & Quideau, 2013; Yoshitake *et al.*, 2016), eventually resembling communities found in natural conditions (Hahn & Quideau, 2013).

Early results from Hanson and Campbell (Chapter 2) determined that our experimental mixtures resembled several physical and chemical aspects of the Attawapiskat River floodplain and shared similar biological properties with the South OB stockpile mix. These early results seem to show the potential of our experimental mixtures to resemble an early successional environment similar to the Attawapiskat River floodplain, able to host native upland plant communities and eventually developing similar microbial activity, and potentially similar communities. Our field and incubation study results might also indicate the potential success of our experimental mixtures to be used as technosols.

Current research suggests that differences in microbial community composition exist between soils that are non-serpentine and serpentine (Oline, 2006; Rajakaruna *et al.*, 2009). Furthermore, differences noted in the respiration rates between our mixtures, and when compared to the natural environment could be attributed to differences in microbial communities with varying functions. Changes in microbial communities have been related to the dynamics of nutrient cycling and plant communities (Batten *et al.*, 2006). Further studies could be conducted to explore the microbial communities that are present within each of these soils. Microbial community research would provide insight into plant-microbial relationships between our non-serpentine and serpentine mixtures, in addition to examining how communities inhabiting our experimental mixtures compare to a natural environment such as the Attawapiskat River.

Conclusion

Our study found no difference in microbial activity between non-serpentine and serpentine technosol mixtures, suggesting processed kimberlite did not inhibit microbial communities in the long-term, over the weeklong incubation experiment. Only short-lived trends in respiration rates were noted between the mixtures increasing along the serpentine gradient, potentially due to minor physical and chemical differences. These short-lived results could support the findings of our field study (Chapter 2). These results could suggest that our non-serpentine mixtures may not only possess more beneficial properties for vegetation establishment, but may also provide slightly better conditions for microbial communities, at least based on the short-term results of our study. Our results could suggest microbial activity did not influence the differences in vegetation establishment found in our field study (Chapter 2). In our study the microbial communities responded positively to the addition of glucose acting as a proxy for soluble carbon naturally released from plant roots in the environment. Microbial communities also responded to positively to the application of more peat, however we were unable to suggest confidently that the differences in microbial activity between our 20% and 40%

peat applications were actually due to different peat applications we tried to achieve based on results from our field study (Chapter 2).

Compared to a natural upland environment undergoing early succession, and a peat-overburden mixture currently being used to rehabilitate a stockpile at the Victor Mine, our experimental mixtures had different microbial activity. In the short-term they shared more similarities to the Victor Mine peat-overburden mixture the short-term, while having lower microbial activity than the river floodplain soil. These results could indicate that the natural environment possesses more microbial communities of greater diversity. The noted early similarities between our mixtures and the natural environment, as determined by Hanson and Campbell (Chapter 2), could indicate the potential of our mixtures to eventually possessing similar microbial activity, and potentially similar microbial communities, resembling an environment similar to the Attawapiskat river floodplain.

To gain a better understanding of technosols containing diamond mine waste a longer-term study should be conducted on the influence processed kimberlite mine waste has on colonizing microbial communities. In addition, further research focusing on microbial communities should be conducted to address several unanswered questions, including how do microbial communities differ across the serpentine gradient we created? How do these microbial communities influence vegetation establishment on non-serpentine and serpentine mixtures? Finally, how do the microbial communities of our experimental mixtures develop over time and compare to reference site communities?

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Tables and Figures

Table 1. Selected mean characteristics of the mineral and peat substrates used to create our experimental mixtures, displaying total and bioavailable elements with their detection limits (DL). Environmental Standards according to CCME industrial soil quality guidelines are shown. Total C, N and S, were gathered by this study, while the remaining total elements were supplied by Bergeron and Campbell (unpublished). Bioavailable elements (A) were provided by Bergeron & Campbell (unpublished) while bioavailable elements (B) were determined for our study.

				Total				Bioavailable (A)					Bioavailable (B)				
	Units	Standards	DL	OB	CPK	FPK	Peat	DL	OB	CPK	FPK	Peat	DL	OB	CPK	FPK	Peat
Texture				silty loam	coarse sand	loamy sand	-										
pH				7.8-8.3	7.98-8.8	8.3-8.8	5										
EC	µs cm^{-1}			352	142	331	331										
C	µg C g^{-1}			10173	28978	37962	109828										
N	µg g^{-1}		100	400	5600	100	150										
S	µg g^{-1}		100	325	2320	105	155										
K	µg g^{-1}		0.1	194168	16661	15593	19929	0.1	1232	134	328	1338	10000	30500	20000	32500	25000
P	µg g^{-1}		0.18	118	384	516	15	0.18	2	<0.18	<0.18	1	5000	<5000	<5000	<5000	<5000
Ca	µg g^{-1}		0.3	1097324	518902	540576	257998	0.3	7561	1033	1308	17726	5000	270500	133500	104300	749000
Mg	µg g^{-1}		1	200659	519119	511674	21308	1	682	1159	1514	3544	400	48900	64450	70200	79700
Na	µg g^{-1}		0.01	133345	37149	26746	24963	0.01	1089	80	1142	1178	10000	51000	<10000	73500	31000
Fe	µg g^{-1}		0.02	152041	386412	321255	66109	0.02	427	1	1	206	2000	<2000	<2000	<2000	<2000
Ni	µg g^{-1}	50	0.02	169	11391	10403	7	0.02	1	0	0	1	100	<100	<100	<100	<100
Mn	µg g^{-1}		0.007	3120	7994	6247	617	0.007	27	<0.007	<0.007	26	100	200	<100	<100	250
Cr	µg g^{-1}	64	0.04	221	9821	4907	<0.04	0.04	0	2	6	0	100	<100	<100	<100	<100
Cu	µg g^{-1}		0.02	102	335	543	<0.02	0.02	3	0	<0.02	1	100	290	<100	<100	290
Co	µg g^{-1}		0.02	85	754	719	10	0.02	0	<0.02	0	<0.02	10	<10	<10	<10	<10
Se	µg g^{-1}	1	0.37	4	882	440	<0.37	0.37	1	2	<0.37	<0.37	100	<100	<100	<100	<100

Table 2. Selected mean bioavailable elements of the South OB stockpile and Attawapiskat River floodplain reference sites, displaying total and bioavailable elements with their detection limits (DL). Environmental Standards according to CCME industrial soil quality guidelines are shown.

	Units	Standards	DL	South OB stockpile	Attawapiskat River
total C	µg C g^{-1}			44834	27845
total N	µg g^{-1}		100	5800	2000
total S	µg g^{-1}		100	1350	980
K	µg g^{-1}		10000	25500	23333
P	µg g^{-1}		5000	<5000	7000
Ca	µg g^{-1}		5000	654167	382667
Mg	µg g^{-1}		400	55050	30067
Na	µg g^{-1}		10000	41833	20000
Fe	µg g^{-1}		2000	<2000	5067
Ni	µg g^{-1}	50	100	<100	1060
Mn	µg g^{-1}		100	752	1533
Cr	µg g^{-1}	64	100	<100	<100
Cu	µg g^{-1}		100	<100	275
Co	µg g^{-1}		10	37	59
Se	µg g^{-1}	1	100	<100	<100

Table 3. Analysis of variance results to compare the CO_2 respiration rates of the mineral mixtures with the factorial addition of peat and glucose. Separate analyses were done for each day.

	Day 0			Day 3			Day 7		
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Block	2	4.756	0.011*	2	0.161	0.851	2	0.068	0.934
Waste Site	1	1.316	0.255	1	6.122	0.016*	1	0.879	0.351
Mineral Mix	3	3.813	0.013*	3	8.181	<0.001***	3	0.734	0.535
% Peat	1	8.124	0.006**	1	7.892	0.006**	1	3.510	0.065*
Glucose	1	3.215	0.077	1	29.501	<0.001***	1	57.809	<0.001***
Mineral Mix x % Peat	3	0.081	0.970	3	1.307	0.278	3	0.663	0.577
Mineral Mix x Glucose	3	0.236	0.871	3	0.549	0.650	3	2.082	0.109
% Peat x Glucose	1	0.109	0.742	1	0.150	0.699	1	0.335	0.565
Mineral Mix x % Peat x Glucose	3	1.128	0.343	3	0.658	0.580	3	0.089	0.966
Error	74			77			77		

Table 4. Analysis of variance to compare the CO_2 respiration rates of the field experimental mixtures and the early successional reference sites; South OB stockpile and the Attawapiskat River floodplain, with the factorial addition of glucose. Separate analyses were done for each day.

	Day 0			Day 3			Day 7		
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Site	2	364.99	<0.001***	2	28.209	<0.001***	2	9.602	<0.001***
Glucose	1	181.119	<0.001***	1	1.026	0.313	1	30.073	<0.001***
Site x Glucose	2	165.148	<0.001***	2	3.686	0.028*	2	9.741	<0.001***
Error	99			102			102		

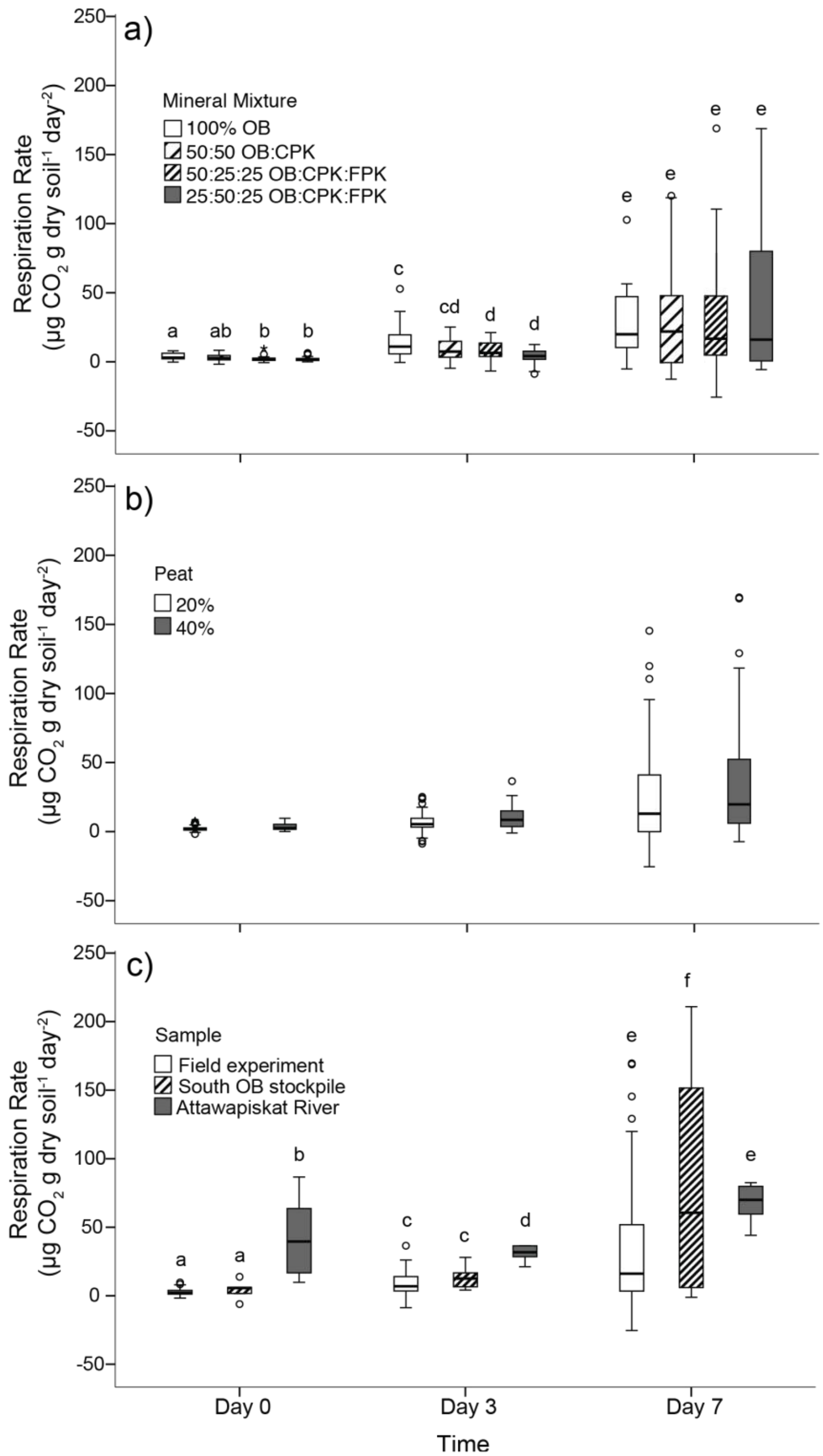

Figure 1. Box plots representing CO_2 respiration rate on day 0, 3, and 7, after 6 hours of incubation, for the a) main effect of mineral mixtures, b) main effect of peat applications, and c) the comparison of the field experiment mixtures with those from two early successional reference sites, the South OB Stockpile and Attawapiskat River floodplain. Post-hoc Tukey test results are shown.

CHAPTER 4: Practical Implications of this Research

Site rehabilitation in remote settings has become more common as mines develop more frequently in northern regions, often involving constructing technosols from available mine waste substrates. Our research will provide valuable information for the rehabilitation of diamond mines, in particular at the De Beers Victor Mine, as well as for the rehabilitation of subarctic disturbances. We constructed technosols with mineral and organic mine waste substrates at the De Beers Victor Mine. Our technosols contained a gradient of mineral matrices, varying from a marine calcareous overburden to having increasing coarse and fine ultramafic processed kimberlite. To our technosols we applied peat as the organic substrate, added fertilizer, and introduced microbial and native plant communities. Our study takes place over a two-year period and only provides us with early results. This research would benefit from longer-term studies to gain a better understanding of how technosols containing diamond mine waste influence colonization. However, our early findings provide insight into the potential successes and limitations of using these substrates.

MINERAL SUBSTRATES

Physical and chemical properties of the mineral mine waste substrates incorporated into technosols can influence influence the colonization of plant and microbial communities. Our research will provide the De Beers Victor Mine with suggestions for technosols to use during rehabilitation and provides insight into the potential limitations of incorporating processed kimberlite into their cover mixtures. In both of our studies, the technosols containing a mineral component of dominantly a silty loam marine overburden provided better conditions than mixes containing processed kimberlite. This was reflected in plant establishment and microbial activity. Currently, the De Beers Victor Mine is using a mixture of peat and a silty loam marine calcareous overburden for rehabilitation. Our peat-overburden mixtures were apparently similar to substrates along the Attawapiskat River, which support diverse vegetation. The success of our peat-overburden mixtures over two years suggests that the peat-overburden mixture being used by De Beers Victor Mine will eventually provide a successful substrate for ecosystem rehabilitation.

Incorporating processed kimberlite into technosols provided limitations, although apparently minor, for plant and microbial colonization. These limitations were related to coarser textures of CPK, the lack of essential nutrients (N, P), elevated levels of other nutrients (Mg), and alkaline conditions in processed kimberlite. We found technosol mixtures containing a mineral component of dominantly silty loam overburden, with non-serpentine characteristics, provided better conditions for early plant colonization than technosols containing processed kimberlite, with serpentine characteristics. However, a remaining problem to be considered may be the binding of calcium and phosphorus to create insoluble complexes of phosphorus, unavailable to colonizing species, as a result of the alkaline nature of all of our technosol mixtures. We found that the reference site conditions in our experiment were naturally quite alkaline, slightly less than our mixtures, therefore only a minor drop in pH may be required.

Throughout our research we gained knowledge about the sensitivity of plant and microbial communities to serpentine characteristics. Our findings were similar in some ways to previous research on natural serpentine soils (Walker, 1954; Brooks, 1987; Brady *et al.*, 2005). Therefore, our research could also contribute broadly to studies on natural serpentine soils and other technosols with serpentine characteristics. In other aspects, our findings were different than studies on natural serpentine soils, such as the levels of some bioavailable of heavy metals in our serpentine mixtures. Therefore, our research may also provide insight into differences between processed kimberlite technosols, technosols consisting of other types of ultramafic waste material, and natural serpentine soils forming from other ultramafic rock types. To gain a better understanding of how technosols containing diamond mine waste influence plant establishment, longer-term studies should be conducted, and also examine physiological influences on establishing communities.

PEAT AMENDMENT

Using peat as an organic amendment in technosols is a common practice in the Athabasca Oil Sands mining developments for the benefits it has on improving the physical and chemical aspects of technosols (Danielson *et al.*, 1983; Rowland *et al.*, 2009). Peat-overburden mixes of 50 to 75% peat content are commonly applied at the surface to a thickness of 20 to 50 cm (Rowland *et al.*, 2009). We chose to examine 20% and 40% peat applications in our study to better understand how the De Beers Victor Mine can use their available organic material effectively and provide reccomendations. In our experiment the incorporation of 20% versus 40% peat did not appear to significantly alter the substrates, although there may have been issues during our technosol construction. Since our application of peat was less than peat generally applied to peat-mineral mixes, and were still successful at establishing native vegetation, we speculate that rehabilitation may potentially be successful even with minimum peat applications. In general, our findings were similar to previous studies involving peat-mineral mine waste technosols used in the Athabasca Oil Sands mining developments which demonstrate the need for incorporating organic matter such as peat into the technosol (Danielson *et al.*, 1983; Rowland *et al.*, 2009). Therefore, our study can contribute to the research being conducted on peat-mineral technosols. Although we did not find differences in plant establishment between our peat applications, our findings still indicate the beneficial properties of using peat as an organic amendment in technosols. Differences in plant establishment between our technosols and mineral mine waste substrate controls, which lacked organic matter, could have been related to peat applications. Our research also demonstrated the beneficial properties of peat for benefitting microbial communities.

FERTILIZATION

A common issue to overcome during rehabilitation is infertility. We applied a mild dose of fertilizer to our technosols and found our mixes were deficient in essential nutrients. Our study did not examine different application rates of fertilizer, so we are unable to make recommendation from our research an application rate that would benefit plant and microbial colonization. However, our research provides insight into infertility challenges that the De Beers Victor Mine may encounter during rehabilitation, such as a potential deficiency in phosphorus, potentially due to the binding nature it may have with calcium in the alkaline mineral mine waste substrates. Further research would need to be conducted to determine the extent essential nutrients were limiting colonization of plant and microbial communities across our serpentine gradient. Research suggests certain microbial communities are capable of improving soil fertility, specifically by acquiring unavailable phosphorus from the environment (Read *et al.*, 2004; Smits *et al.*, 2012; Taktek *et al.,* 2015). In order to gain a better understanding of nutrient limitations in non-serpentine and serpentine technosols further research into fertilization and phosphate solubilizing microbial communities should be conducted.

MICROORGANISIMS

Soil microbial communities are recognized as important indicators for soil function and are also important to consider during rehabilitation. We did not test our technosols without the addition of a microbial inoculation mixture, so we are unable to determine the extent microbial communities may have influenced plant establishment between our non-serpentine and serpentine technosols to provide specific recommendations to the De Beers Victor Mine. However, based on previous research we suspect that introducing microbial communities from local forest litter is important during rehabilitation (McMillan *et al.,* 2007; Larney & Angers, 2012; Hahn & Quideau, 2013), therefore may have been effective at introducing these communities to our mixtures. Differences in microbial activity between our non-serpentine and serpentine technosols were minimal, and only minor during part of our experiment. Further research would need to be conducted to gain a better understanding of how technosols containing diamond mine waste influence microbial communities, including distribution and physiological influences. This study would benefit from research conducted specifically on microbial communities to determine if they differ between non-serpentine and serpentine technosols, and to what extent the communities are similar to those from early successional reference environments. In general, our study provided insight into factors that can limit microbial activity by examining how microbes are influenced by soluble carbon within the soil and provided insight into the benefits of using peat as an organic amendment to improve microbial activity.

MICROCLIMATE MODERATIONS

One of the main challenges encountered in our study was the remote location of the De Beers Victor Mine, limiting the available substrates for rehabilitation. We were able to demonstrate that technosols built from local substrates provide conditions conducive for the colonization of microbes and plants, at least over two years. The subarctic climate of the De Beers Victor Mine also provided challenging conditions for early plant and microbial colonization due to shorter growing seasons, potential for needle-ice formation, and fewer effective growing degree-days than locations further south. Beyond the Victor Mine, our research will contribute to the current understanding of subarctic ecosystem rehabilitation following severe disturbances. It could be useful for future developments in Ontario's Far North, such as in the proposed Ring of Fire and be valuable for subarctic regions globally.

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Appendix

Table A1. Selected mean characteristics of the mineral and peat substrates used to create our experimental mixtures, displaying total and bioavailable elements with their detection limits (DL). Environmental Standards according to CCME industrial soil quality guidelines are shown. Total C, N, S, and LOI total C were gathered by this study, while the remaining total elements were supplied by Bergeron and Campbell (unpublished). Bioavailable elements (A) were provided by Bergeron & Campbell (unpublished) while bioavailable elements (B) were determined for our study.

				Total				Bioavailable (A)					Bioavailable (B)				
	Units	Standards	DL	OB	CPK	FPK	Peat	DL	OB	CPK	FPK	Peat	DL	OB	CPK	FPK	Peat
Texture				silty loam	coarse sand	loamy sand	-										
pH				7.8-8.3	7.98-8.8	8.3-8.8	5										
EC	$\mu s\ cm^{-1}$			352	142	331	331										
LOI total C	$\mu g\ C\ g^{-1}$			10173	28978	37962	109828										
C	$\mu g\ g^{-1}$		100	49250	120400	31250	29100										
N	$\mu g\ g^{-1}$		100	400	5600	100	150										
S	$\mu g\ g^{-1}$		100	325	2320	105	155										
K	$\mu g\ g^{-1}$		0.1	194168	16661	15593	19929	0.1	1232	134	328	1338	10000	30500	20000	32500	25000
P	$\mu g\ g^{-1}$		0.18	118	384	516	15	0.18	2	<0.18	<0.18	1	5000	<5000	<5000	<5000	<5000
Ca	$\mu g\ g^{-1}$		0.3	1097324	518902	540576	257998	0.3	7561	1033	1308	17726	5000	270500	133500	104300	749000
Mg	$\mu g\ g^{-1}$		1	200659	519119	511674	21308	1	682	1159	1514	3544	400	48900	64450	70200	79700
Na	$\mu g\ g^{-1}$		0.01	133345	37149	26746	24963	0.01	1089	80	1142	1178	10000	51000	<10000	73500	31000
Fe	$\mu g\ g^{-1}$		0.02	152041	386412	321255	66109	0.02	427	1	1	206	2000	<2000	<2000	<2000	<2000
Ni	$\mu g\ g^{-1}$	50	0.02	169	11391	10403	7	0.02	1	0	0	1	100	<100	<100	<100	<100
Mn	$\mu g\ g^{-1}$		0.007	3120	7994	6247	617	0.007	27	<0.007	<0.007	26	100	200	<100	<100	250
Cr	$\mu g\ g^{-1}$	64	0.04	221	9821	4907	<0.04	0.04	0	2	6	0	100	<100	<100	<100	<100
Cu	$\mu g\ g^{-1}$		0.02	102	335	543	<0.02	0.02	3	0	<0.02	1	100	290	<100	<100	290
Co	$\mu g\ g^{-1}$		0.02	85	754	719	10	0.02	0	<0.02	0	<0.02	10	<10	<10	<10	<10
Se	$\mu g\ g^{-1}$	1	0.37	4	882	440	<0.37	0.37	1	2	<0.37	<0.37	100	<100	<100	<100	<100

Table A2. Analysis of variance table for mean percent organic matter lost for experimental mixtures.

Source	df	*F*	*P*
Block	1	0.251	0.622
Mineral Mix	3	0.980	0.420
Peat	1	0.008	0.929
Tops VS Sides	1	0.452	0.509
Mineral Mix x Peat	3	0.200	0.895
Error	22		

Table A3. Analysis of variance table for mean bulk density (g cm^{-3}) of experimental mixtures.

Source	df	*F*	*P*
Block	2	8.725	0.001***
Waste Site	1	2.041	0.159
Mineral Mix	3	3.398	0.024*
Peat	1	0.718	0.401
Tops VS Sides	1	1.812	0.184
Mineral Mix x Peat	3	0.908	0.443
Error	52		

Table A4. Analysis of variance table for mean root penetrability resistance (N cm^{-1}) measurements of experimental mixtures from 5 cm to 20 cm depth, in 5 cm increments.

Source	df	*F*	*P*
Block	1	1.326	0.333
Mineral Mix	3	1.220	0.437
Peat	1	0.005	0.953
Mineral Mix x Peat	3	0.425	0.750
Error a	3		
Depth	4	0.504	0.739
Depth x Mineral Mix	12	1.613	0.210
Error b	12		

Table A5. Analysis of variance tables for mean surface roughness on the experimental mixtures, measured in June 2015 and 2016.

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Block	1	14.115	0.007**	1	2.886	0.133
Mineral Mix	3	5.471	0.030*	3	0.639	0.614
Peat	1	0.117	0.743	1	1.589	0.248
Mineral Mix x Peat	3	1.634	0.266	3	0.261	0.851
Error	7			7		

Table A6. Analysis of variance table for mean volumetric water content (m^3 m^{-3}) from June to August 2015, for the surface and sides of the experimental mixtures.

Source	df	*F*	*P*
Block	2	0.336	0.715
Waste Site	1	92.94	<0.001***
Mineral Mix	3	7.302	<0.001***
Peat	1	3.17	0.077
Top VS side	1	2.405	0.123
Date	2	0.645	0.526
Mineral Mix x Peat	3	1.011	0.389
Error	178		

Table A7. Analysis of variance table for mean volumetric water content (m^3 m^{-3}) of the surface and sides of the experimental mixtures, measured 24, 48, and 72 hours after a rainfall event.

Source	df	*F*	*P*
Block	1	68.087	<0.001***
Mineral Mix	3	4.449	0.057*
Peat	1	0.316	0.575
Top VS Side	1	0.432	0.512
Hours	2	4.767	0.058*
Mineral Mix x Peat	3	0.344	0.794
Mineral Mix x Hours	6	2.475	0.027*
Error	126		

Table A8. Maximum and minimum temperatures (^{o}C) recorded from July 22, 2015 to June 5, 2016 at various depths within the profile of the 100% OB-20% peat and 50:50 OB:CPK-20% peat mixtures, South OB stockpile, and Attawapiskat River soil.

Site		3cm	5cm	10cm	15cm	20cm
100% OB	MAX	41	35.6	31.8	28.4	25.8
	MIN	-20.8	-20.4	-18.5	-17.3	-16.5
50:50 OB:CPK	MAX	43.2	44.2	36.9	30.2	26.6
	MIN	-24.4	-24.9	-22	-18.2	-15.3
South OB stockpile	MAX	34	31.5	22.2	18.9	16.3
	MIN	-17.6	-17.3	-13.4	-12.1	-11.1
Attawapiskat River	MAX	28	25.1	22.7	21	21.3
	MIN	-2.1	-1.1	-0.7	-0.4	-4.3

Table A9. Analysis of variance table for mean surface pH of experimental mixtures in June and August 2015.

Source	df	*F*	*P*
Block	1	0.408	0.588
Waste Site	2	0.373	0.729
Mineral Mix	3	4.257	0.023*
Peat	1	0.009	0.930
Mineral Mix x Peat	3	1.055	0.397
Error a	15		
Date	1	41.993	0.001**
Date x Mineral Mixture	3	2.758	0.079
Error b	15		

Table A10. Analysis of variance table for mean surface electrical conductivity (µs cm^{-1}) of the experimental mixtures in June and August 2015.

Source	df	*F*	*P*
Block	2	2.053	0.328
Waste Site	1	22.910	0.041*
Mineral Mix	3	4.839	0.015*
Peat	1	2.500	0.175
Mineral Mix x Peat	3	0.502	0.686
Error a	15		
Date	1	1.109	0.340
Date x Mineral Mix	3	7.143	0.003**
Error b	15		

Table A11. Permutational multivariate analysis of variance table for bioavailable elements in the experimental mixtures in 2014 and 2015.

Source	df	Pseudo-F	P (perm)
Block	2	1.776	0.123
Waste Site	1	14.389	<0.001***
Mineral Mix	3	11.058	<0.001***
Peat	1	5.168	0.007
Mineral Mix x Peat	3	0.402	0.946
Error a	37		
Whole-plot	47	3.253	<0.001***
Year	1	11.771	<0.001***
Year x Mineral Mix	3	0.365	0.968
Year x Peat	1	0.385	0.808
Year x Mineral Mix x Peat	3	0.716	0.699
Error b	40		

Table A12. Selected mean bioavailable elements of the experimental mineral-peat soil mixtures from 2014 and 2015.

Peat			20%				40%			
Element	Units	DL	100% OB	50:50 OB:CPK	50:25:25 OB:CPK:FPK	25:50:25 OB:CPK:FPK	100% OB	50:50 OB:CPK	50:25:25 OB:CPK:FPK	25:50:25 OB:CPK:FPK
K	$\mu g\ g^{-1}$	10000	34917	26000	23727	22833	31500	24583	25955	20917
P	$\mu g\ g^{-1}$	5000	6000	<5000	<5000	<5000	<5000	8000	<5000	<5000
Ca	$\mu g\ g^{-1}$	5000	435667	354833	352417	298500	391667	318000	296333	277333
Mg	$\mu g\ g^{-1}$	400	63025	80158	76775	82483	55600	71608	77942	77250
Na	$\mu g\ g^{-1}$	10000	14667	14000	18400	15000	20000	14444	23700	12857
Fe	$\mu g\ g^{-1}$	2000	<2000	5267	<2000	<2000	5000	<2000	<2000	<2000
Ni	$\mu g\ g^{-1}$	100	100	242	129	133	100	129	188	125
Mn	$\mu g\ g^{-1}$	100	347	267	234	192	359	206	192	160
Cr	$\mu g\ g^{-1}$	100	<100	<100	<100	<100	<100	<100	<100	100
Cu	$\mu g\ g^{-1}$	100	184	249	157	188	165	180	319	270
Co	$\mu g\ g^{-1}$	10	10	30	13	13	10	24	30	31
Se	$\mu g\ g^{-1}$	100	<100	<100	<100	<100	<100	<100	<100	<100

Table A13. Analysis of variance tables for mean total plant cover on the surface and sides of the experimental mixtures in August 2015 and 2016.

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Block	2	0.106	0.899	2	1.088	0.34
Waste Site	1	66.737	<0.001***	1	7.986	0.01**
Mineral Mix	3	6.515	0.001***	3	10.236	<0.001***
Peat	1	0.727	0.398	1	0.537	0.47
Tops VS Sides	1	4.748	0.034*	1	4.003	0.05
Mineral Mix x Peat	3	0.067	0.977	3	2.142	0.11
Error	52			56		

Table A14. Analysis of variance tables for above ground biomass (g cm^{-2}; Log_{10} transformation) on the experimental mixtures in August 2015 and 2016.

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Block	2	2.788	0.075	2	0.094	0.911
Waste Site	1	13.13	0.001***	1	0.004	0.950
Mineral Mix	3	2.14	0.112	3	1.427	0.258
Peat	1	0.573	0.454	1	0.283	0.599
Mineral Mix x Peat	3	0.84	0.481	3	1.034	0.394
Error	37			25		

Table A15. Analysis of variance tables for plant species richness (Log_{10} transformation) on the surface and sides of the experimental mixtures in August 2015 and 2016.

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Block	2	1.558	0.22	2	4.53	0.015*
Waste Site	1	6.386	0.015*	1	11.189	0.001***
Mineral Mix	3	0.954	0.421	3	1.393	0.254
Peat	1	0.026	0.872	1	0.252	0.618
Top VS Sides	1	41.45	<0.001***	1	84.755	<0.001***
Mineral Mix x Peat	3	0.733	0.537	3	0.126	0.944
Error	52			57		

Table A16. Analysis of variance tables for percent organic matter lost of a) all experimental mixtures in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile.

a)

Source	df	*F*	*P*
Site	4	31.779	<0.001***
Sample Number	79	0.804	0.805
Error	46		

b)

Source	df	*F*	*P*
Site	2	17.346	0.001***
Sample Number	19	0.928	0.581
Error	8		

Table A17. Analysis of variance tables for bulk density (g cm^{-3}) of a) all experimental mixture surfaces in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile.

a)

Source	df	*F*	*P*
Site	4	7.419	<0.001***
Sample Number	235	0.608	0.988
Error	41		

b)

Source	df	*F*	*P*
Site	2	45.158	<0.001***
Sample Number	59	1.916	0.165
Error	8		

Table A18. Analysis of variance tables for root penetrability resistance (N cm^{-1}) of a) all experimental mixture surfaces in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile.

a)

Source	df	*F*	*P*
Site	4	61.272	<0.001***
Error a	31		
Depth	4	4.428	0.01
Site x Depth	12	0.763	0.68
Error b	20.352		

b)

Source	df	*F*	*P*
Site	2	1.8	0.226
Error a	8		
Depth	4	0.682	0.609
Site x Depth	8	0.462	0.873
Error b	30.786		

Table A19. Analysis of variance tables for surface roughness of a) all experimental mixture surfaces in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil in June 2015, and experimental mixtures in comparison to one plot on the South OB stockpile and river in June 2016, and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile in June 2015.

a)

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Site	4	10.625	<0.001***	2	0.287	0.777
Sample Number	31	0.442	0.989	31	1.25	0.542
Error	38			2		

b)

	2015		
Source	df	*F*	*P*
Site	2	0.298	0.753
Sample Number	9	0.679	0.711
Error	6		

Table A20. Analysis of variance tables for surface volumetric water content (m^3 m^{-3}) from June to August 2015 of a) all experimental mixture surfaces in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile.

a)

Source	df	*F*	*P*
Site	3	22.261	<0.001***
Sample Number	229	1.139	0.114
Date	13	11.13	<0.001***
Error	577		

b)

Source	df	*F*	*P*
Site	1	1.989	0.161
Sample Number	59	0.627	0.978
Date	4	2.509	0.045*
Error	140		

Table A21. Analysis of variance tables for pH in early June and late August 2015 of a) pH of all experimental mixture surfaces and with depth measurements in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, b) surface pH and pH with depth of our 100% OB-peat mixture in comparison to the East OB stockpile and c) surface pH and pH with depth of our 100% OB-peat mixtures in comparison to the East OB stockpile over the two sample dates (June and August 2015).

a)

Source	df	*F*	*P*
Site	4	11.362	<0.001***
Error a	36		
Depth	1	8.779	0.053*
Site x Depth	2	1.961	0.161
Error b	25.818		
Date	2	10.850	0.032*
Site x Date	3	17.430	<0.001***
Site x Date x Depth	2	0.123	0.885
Depth x Date	1	1.369	0.260
Error c	15.603		

b)

Source	df	*F*	*P*
Site	2	15.903	<0.001***
Sample Number	47	2.120	0.001***
Depth	1	0.042	0.839
Error	85		

c)

Source	df	*F*	*P*
Site	2	22.078	<0.001***
Sample Number	47	3.083	<0.001***
Date	1	22.064	<0.001***
Error	85		

Table A22. Analysis of variance tables for electrical conductivity (μs cm^{-1}) in early June and late August 2015 of a) all experimental mixture surfaces and with depth measurements in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, b) surface electrical conductivity, and electrical conductivity with depth of our 100% OB-peat mixture in comparison to the East OB stockpile and c) surface electrical conductivity, and electrical conductivity with depth in our 100% OB-peat mixtures in comparison to the East OB stockpile over the two sample dates (June and August 2015).

a)

	df	*F*	*P*
Site	4	9.687	<0.001***
Error a	36		
Depth	1	1.800	0.255
Site x Depth	2	1.916	0.167
Error b	26.313		
Date	2	1.387	0.269
Site x Date	3	0.994	0.410
Site x Date x Depth	2	0.285	0.755
Depth x Date	1	1.609	0.217
Error c	24.334		

b)

	df	*F*	*P*
Site	2	13.932	<0.001***
Sample Number	47	0.654	0.943
Depth	1	1.513	0.222
Error	84		

c)

	df	*F*	*P*
Site	2	14.942	<0.001***
Sample Number	47	0.689	0.917
Date	1	4.542	0.036*
Error	84		

Table A23. Selected mean bioavailable elements of the WR dump, PK Cell cover mix, South OB stockpile, and Attawapiskat River floodplain reference sites, with their detection limits (DL).

	Units	DL	WR dump	PK Cell cover mix	East OB	South OB	Attawapiskat River
LOI total C	µg C g^{-1}					44834	27844
total C	µg g^{-1}	100				145100	49800
total N	µg g^{-1}	100				5800	2000
total S	µg g^{-1}	100				1350	980
K	µg g^{-1}	10000	41200	20000	56000	25500	23333
P	µg g^{-1}	5000	<5000	<5000	<5000	<5000	7000
Ca	µg g^{-1}	5000	209000	896200	273200	654167	382667
Mg	µg g^{-1}	400	35140	60540	59620	55050	30067
Na	µg g^{-1}	10000	23333	20000	81600	41833	20000
Fe	µg g^{-1}	2000	<2000	<2000	<2000	<2000	5067
Ni	µg g^{-1}	100	<100	<100	<100	<100	1060
Mn	µg g^{-1}	100	<100	608	265	752	1533
Cr	µg g^{-1}	100	660	<100	<100	<100	<100
Cu	µg g^{-1}	100	<100	200	200	<100	275
Co	µg g^{-1}	10	<10	<10	<10	37	59
Se	µg g^{-1}	100	<100	<100	<100	<100	<100

Table A24. Analysis of variance tables for mean total plant cover of a) all experimental mixture surfaces in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile in 2015.

a)

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Site	4	17.995	<0.001***	4	28.007	<0.001***
Sample Number	48	0.613	0.945	37	0.649	0.898
Error	37			32		

b)

Source	df	*F*	*P*
Site	2	14.456	0.002**
Sample Number	11	1.141	0.437
Error	8		

Table A25. Analysis of variance tables for plant species richness (Log_{10} transformation) of a) all experimental mixture surfaces in comparison to the WR dump, PK Cell cover mix, South OB stockpile, and river soil, and b) our 100% OB-peat mixture surfaces in comparison to the East OB stockpile in 2015.

a)

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Site	3	35.766	<0.001***	4	26.158	<0.001***
Sample Number	57	0.302	1.000	38	0.269	1.000
Error	27			32		

b)

Source	df	*F*	*P*
Site	2	13.794	<0.001***
Sample Number	11	1.145	0.435
Error	8		

Table A26. Analysis of variance tables for above ground biomass (g cm^{-2}; Log_{10} transformation) on all experimental mixtures in comparison to the South OB stockpile and Attawapiskat River floodplain in August 2015 and 2016.

	2015			2016		
Source	df	*F*	*P*	df	*F*	*P*
Site	2	1.165	0.321	2	0.626	0.540
Error	46			36		

Table A27. Plant species assemblages contributing to the average dissimilarity between experimental mixtures and the Attawapiskat River floodplain during August 2015 and 2016. Values were obtained from SIMPER analyses.

	River Floodplain	Field Experiment		
Plant Species	Avg. Abundance	Avg. Abundance	Contribution (%)	Cumulative Contribution (%)
Grasses (unidentifiable)	3.88	0.18	6.03	6.03
Poa palustris	0.00	2.76	4.51	10.54
Dasiphora fructicosa	2.73	0.00	4.44	14.98
Viola adunca	2.22	0.00	3.37	18.35
Asteraceae sp.	2.20	0.00	3.36	21.71
Primula mistassinica	1.98	0.00	3.02	24.73
Erysimum cheiranthoides	0.78	2.34	2.95	27.68
Smilacina stellata	1.74	0.00	2.87	30.55
Prenanthes racemosa	1.67	0.00	2.84	33.39
Thalictrum sparsifolium	1.65	0.00	2.81	36.20
Equisetum arvense	2.00	0.67	2.76	38.96
Salix sp.	1.61	0.00	2.55	41.51
Prunella vulgaris	1.67	0.00	2.49	44.00
Agrostis scabra	0.07	1.48	2.32	46.32
Artemisia tilesii	0.00	1.43	2.29	48.61
Fragaria vesca	1.37	0.00	2.15	50.76
Rosa acicularis	1.31	0.00	2.13	52.89
Achillea millefolium	0.48	1.48	2.12	55.00
Vicia americana	1.33	0.00	2.07	57.07
Polygonum fowleri	0.63	1.32	2.05	59.13
Polygala polygama	1.13	0.00	2.05	61.18
Solidago nemoralis	1.25	0.00	1.89	63.07
Gentianella amarella	1.08	0.00	1.73	64.80
Euphrasia arctica	1.00	0.00	1.55	66.35
Polygala paucifolia	0.85	0.00	1.52	67.88
Alnus incana	0.98	0.00	1.45	69.32
Mentha arvensis	0.99	0.00	1.36	70.68
Beckmannia syzigachne	0.00	0.84	1.35	72.03
Epilobium ciliatum	0.54	0.69	1.33	73.37
Lobelia kalmii	0.94	0.00	1.27	74.64
Epilobium angustifolium	0.09	0.75	1.24	75.88
Packera paupercula	0.81	0.00	1.22	77.10
Populus balsamifera	0.76	0.00	1.21	78.31
Hordeum jubatum	0.07	0.72	1.20	79.51
Picea mariana	0.74	0.00	1.12	80.63
Solidago sp.	0.62	0.00	1.05	81.69
Pinguicula vulgaris	0.76	0.00	1.05	82.73
Physocarpus opalifolius	0.63	0.00	1.03	83.76
Betula pumila glandufira	0.63	0.00	1.01	84.78
Polyganum persicaria	0.13	0.59	1.01	85.78

Ranunculus sp.	0.59	0.00	0.95	86.74
Chenopodium album	0.32	0.32	0.93	87.66
Equisetum variegatum	0.61	0.05	0.89	88.56
Erigeron hyssopifolius	0.61	0.00	0.87	89.43
Juncus arcticus var. balticus	0.66	0.00	0.87	90.30
Tofielda glutinosa	0.51	0.00	0.79	91.09
Chenopodium glaucum	0.31	0.22	0.78	91.87
Puccinellia distans	0.41	0.00	0.74	92.61
Potentilla norvegica	0.45	0.00	0.66	93.27
Solidago ptarmicoides	0.43	0.00	0.62	93.89
Abies balsamea	0.31	0.00	0.54	94.43
Thalictrum sparsifolium	0.36	0.00	0.52	94.95
Hieracium umbellatum	0.18	0.13	0.49	95.44
Carex aurea	0.37	0.00	0.48	95.92
Galium Boreale	0.33	0.00	0.47	96.40
Rubus ideaus	0.00	0.29	0.47	96.86
Populus tremuloides	0.27	0.00	0.47	97.33
Juncus dudleyi	0.32	0.00	0.40	97.73
Parnassia palustris	0.27	0.00	0.38	98.12
Argentina anserina	0.21	0.00	0.31	98.43
Carex interior	0.20	0.00	0.24	98.67
Populus tremuloides	0.16	0.00	0.23	98.89
Calamagrostis stricta	0.07	0.07	0.22	99.11
Castilleja septentrionalis	0.16	0.00	0.21	99.32
Juncus sp.	0.13	0.00	0.20	99.52
Arnica chamissionis	0.00	0.12	0.20	99.72
Lycopus armericanus	0.11	0.00	0.17	99.89
Taraxacum sp.	0.00	0.04	0.07	99.96
Festuca brachyphylla	0.00	0.02	0.04	100.00

Table A28. Growing degree-days (GDD) using air temperature from July 4 to October 31 2015 and from April 1 to August 23 2016, for the experimental mixtures constructed on the PK Cell and WR dump, the South OB stockpile, and Attawapiskat River floodplain.

	2015	2016
Site	GDD	GDD
PK Cell	737	965
WR dump	898	965
South OB stockpile	885	959
Attawapiskat River	911	979

Table A29. Growing degree-days (GDD) using temperature from 3 cm below the soil surface from July 22 to October 31 2015 and from April 1 to August 23 2016, for the experimental mixtures constructed on the PK Cell and WR dump, the South OB stockpile, and Attawapiskat River floodplain.

	2015	2016
Site	GDD	GDD
PK Cell	801	1200
WR dump	756	1123
South OB stockpile	690	888
Attawapiskat River	496	1097

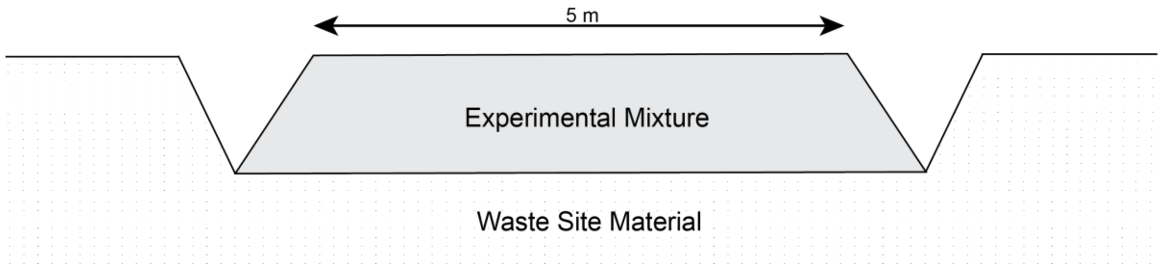

Figure A1. Cross-section diagram depicting the trenches in which our experimental mixtures were constructed, on the WR dump and PK Cell waste sites.

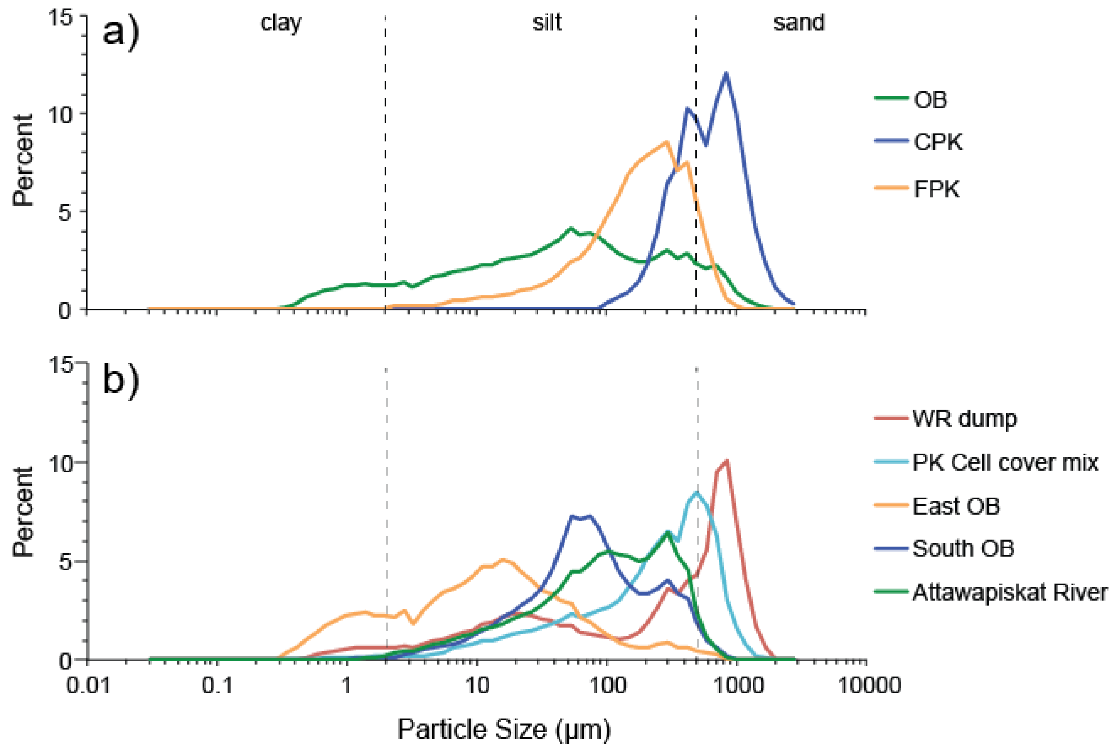

Figure A2. Percent particle sizes (µm) for a) mineral mine waste materials used to construct our experimental mixtures (*n* = 2 each), and b) WR dump, PK Cell cover mix, East OB stockpile, South OB stockpile, and Attawapiskat River floodplain reference sites (*n* = 3 each). We dried the samples at 105 ^{o}C for 24 hours and sieved them through a 2 mm mesh screen. The materials were then analyzed using a Microtrac® S3500 laser diffraction particle size analyzer at the Geoscience Laboratories in Sudbury, ON.

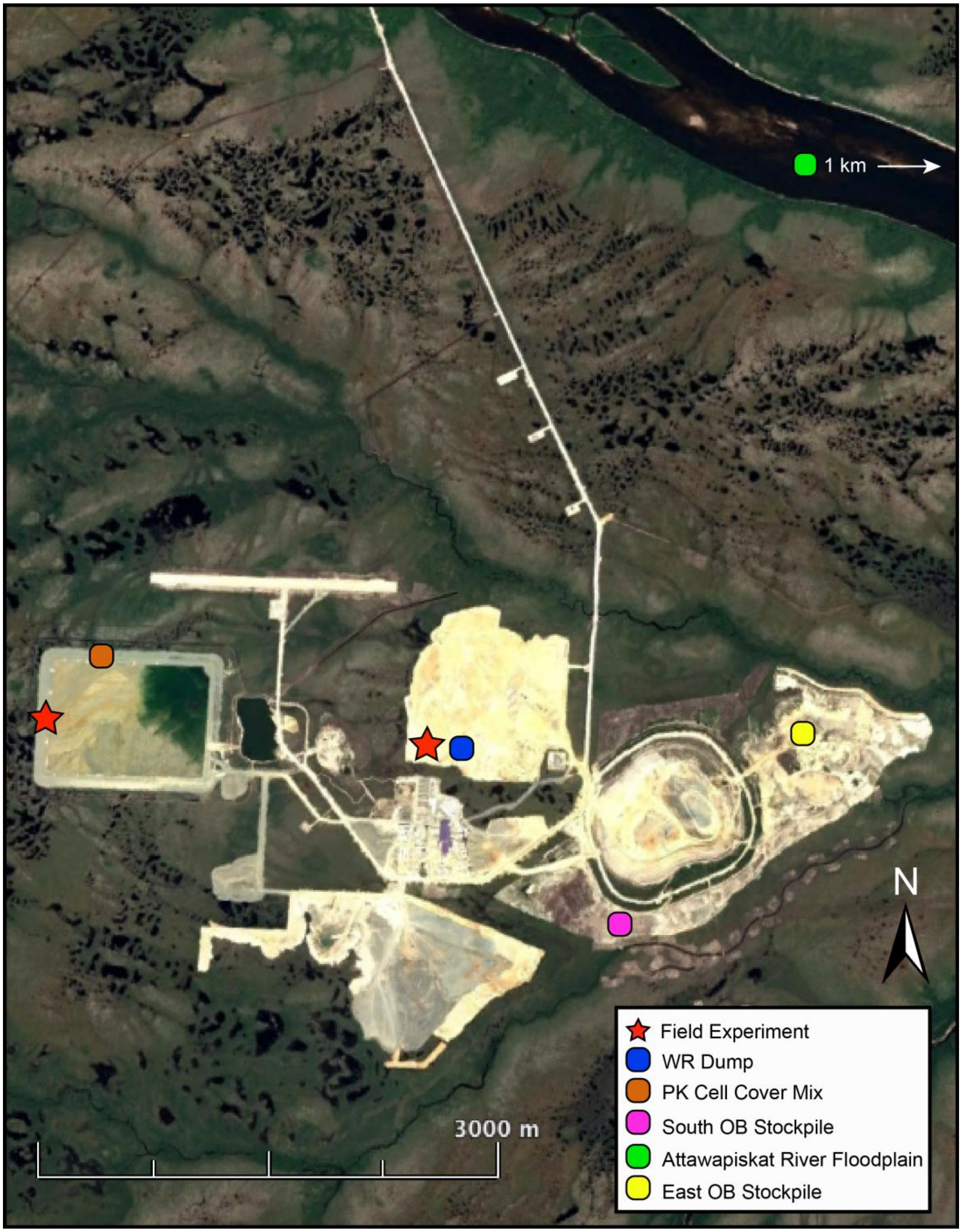

Figure A3. Aerial photograph of the De Beers Victor Mine, Canada, displaying the location of the field experimental plots and reference site sampling points; WR dump, PK Cell cover mix, South OB stockpile, Attawapiskat River floodplain, and East OB stockpile. Image taken on July 6, 2013, obtained from Google Earth.